



PROJECT 4: HATCHERY TROUT EVALUATIONS

Grant # F-73-R-25

Report Period July 1, 2002 to June 30, 2003



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Senior Fisheries Research Biologist

IDFG Report Number 03-44
August 2003

Annual Performance Report

July 1, 2002 to June 30, 2003

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Project 4: Hatchery Trout Evaluations

- Subproject 1: Improving Vulnerability of Rainbow Trout—A Selective Breeding Experiment**
- Subproject 2: Sterile Trout Investigations**
- Subproject 3: Predator Training**

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**IDFG Report Number 03-44
August 2003**

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**ANNUAL PERFORMANCE REPORT
SUBPROJECT #1: IMPROVING VULNERABILITY TO ANGLING OF RAINBOW TROUT—A
SELECTIVE BREEDING EXPERIMENT**

State of: Idaho Grant No.: F-73-R-25 Fishery Research
Project No.: 4 Title: Hatchery Trout Evaluations
Subproject #1: Improving Vulnerability to Angling of
Hatchery Rainbow Trout—A Selective
Breeding Experiment
Contract Period: July 1, 2002 to June 30, 2003

ABSTRACT

A primary goal of this project is to maximize return to creel of stocked fish, thereby improving cost efficiency of IDFG's resident hatchery trout program. I compared the return to creel and number of days to harvest for two groups of catchable-sized rainbow trout *Oncorhynchus mykiss*. The groups were produced from: 1) normal Hayspur-strain broodstock and 2) Hayspur-strain broodstock that exhibited high levels of vulnerability to angling. One-year-old replacement brood fish were uniquely tagged and held in three outdoor raceways. Ninety-four, one hour fishing trials were conducted, and capture frequency for each fish was recorded. Fish caught three or more times were retained for use as broodstock. Equal numbers of progeny from the normal and vulnerable broodstocks were jaw tagged and stocked into 16 waters during 2001 and an additional 16 waters during 2002. Reward incentives, press releases, personal contacts, and signs were used to encourage angler tag reporting in returning tags.

A total of 798 tags were returned out of 6,389 stocked during 2001. Mean first year return rate for the vulnerable group ($12.7 \pm 3.5\%$) was not statistically different from the normal group ($11.7 \pm 3.8\%$). Only 17 tags (2% of the total) were returned from fish caught during the second fishing season. The mean time to harvest was 46.4 ± 9.8 d for the vulnerable group and 50.6 ± 10.7 d for the normal group. This disparity was not statistically different (paired *t*-test, $p = 0.77$). For fish stocked during 2002, 700 tags were returned out of 9,593 stocked. Mean first year return rate for the vulnerable group ($7.2 \pm 2.5\%$) was not different from the normal group ($7.4 \pm 2.7\%$). There was no difference in mean time to harvest for the normal group (36.0 ± 8.0 d) and vulnerable group (38.7 ± 7.3 d; paired *t*-test, $p = 0.45$). No performance benefit in terms of increasing return to creel or reducing time to harvest was achieved through selective breeding.

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INTRODUCTION

The Idaho Department of Fish and Game (IDFG) plants about three million put-and-take rainbow trout *Oncorhynchus mykiss*, subsequently referred to as catchables, annually. Approximately 60% of the catchables are released in lakes, reservoirs and ponds, and the remaining fish are stocked in streams and rivers. The primary objective of the put-and-take program is increasing angling opportunity and harvest. Of the three million catchables stocked every year, anglers harvest about one million (Teuscher et al. 1998). Assuming an optimistic 40% of the catchables are harvested, where do the remaining 60% go and what are the associated costs of producing fish that are not caught? In pure economic terms, the losses account for about \$660,000 and make up 30% of the total resident hatchery budget (IDFG 1998). Fish losses also affect anglers by lowering harvest and catch rates. The substantial loss of catchables begs the question—can return rates be improved (Teuscher et al. 1998)?

The IDFG has completed a number of research projects directed at improving return-to-creel of hatchery rainbow trout. Many of the studies focused on what size of rainbow trout to stock (Culpin 1958; Mauser 1992; Mauser 1994; Teuscher 1999). Other studies evaluated fish behavior (Dillon and Alexander 1996), stocking time, release methods, and fish condition (Casey et al. 1968; Welsh et al. 1970). This report summarizes preliminary results from a three-year study and evaluates the possibility of increasing returns by selecting broodstock that exhibit high levels of angling vulnerability. The success of this project depends on meeting two assumptions. First, individual trout in the Hayspur strain (R9) broodstock population must demonstrate varying degrees of angling vulnerability (Teuscher 2000). Secondly, angling vulnerability must be heritable in this broodstock.

Individual fish exhibit varying degrees of hook-and-line vulnerability. Burkett et al. (1986) reported that largemouth bass *Micropterus salmoides* in Ridge Lake, Illinois demonstrated “high” and “low” angling vulnerability. After four years of fishing effort, the lake was drained and capture frequency for each bass was determined. Out of 1,787 bass examined, 14.3% were never caught, while 19.4% of the bass were caught six or more times. Hackney and Linkous (1978) also reported that largemouth bass populations have easily harvestable segments. In a six-week fishing trial on 94 fish, 23% were never caught, while 21% were caught two or more times. Individuality of angling vulnerability has also been shown for rainbow trout. Lewynsky (1986) observed that during a nine-week fishing trial in a raceway, captures ranged from zero to five times per individual trout. About 37% of the fish were caught more than one time, and 21% were never caught. These studies indicate that some individual fish are more likely than others to be caught by hook-and-line methods, but they give no indication as to the heritability of this trait.

Angling vulnerability may be heritable. Perhaps the most commonly cited studies that link genetic contribution and angling vulnerability are strain evaluations. A common theme among the strain studies is that faster growing strains are more vulnerable to angling, and growth in field tests was generally higher for domesticated or hybrid stocks (Tave et al. 1981; Brauhn and Kincaid 1982; Dwyer and Piper 1984; Nuhfer and Alexander 1994; Yoneyama et al. 1997). Strain effects on angling vulnerability have been demonstrated for largemouth bass (Zolczynski and Davies 1976; Burkett et al. 1986; Kleinsasser et al. 1990), rainbow trout (Brauhn and Kincaid 1982; Moring 1982; Hudy and Berry 1983; Dwyer and Piper 1984), cutthroat trout *O. clarki* (Dwyer 1990), brook trout *Salvelinus fontinalis* (Nuhfer and Alexander 1994), tilapia *Oreochromis* sp. (Yoneyama et al. 1997), and blue catfish *Ictalurus furcatus* (Tave

et al. 1981). Similar mechanisms likely pattern the growth and ultimately the catchability of hatchery rainbow trout.

If angling vulnerability is heritable, then it should be possible to increase returns by selecting broodstock that are vulnerable to angling. In small Texas ponds, angling trials revealed that a largemouth bass population possessed individuals with varying levels of angling vulnerability. Garrett (1993) then selectively bred highly vulnerable males with highly vulnerable females and wary males with wary females. The two groups of progeny were reared separately until age-1, marked, and combined into one pond. In subsequent fishing trials, the catch rate of progeny from the highly vulnerable group was twice that of the progeny from the wary group. With a similar study design, David Phillips (Illinois Natural History Survey, unpublished data) also noted that the catchability of largemouth bass could be altered markedly through selective breeding over several generations.

Angling vulnerability may also differ by sex. Holtby et al. (1992) compared the proportion of juvenile male and female coho *O. kisutch* captured by anglers and in seine hauls. The seine hauls were assumed to be an unbiased representation of the population. Male coho appeared to be more aggressive feeders based upon a proportionally higher return in the recreational fishery than in the seine hauls. On average, males were slightly larger in this study, so differential size may have biased this result. However, the authors speculated that the size difference was produced by more aggressive feeding by males, as males and females were equal in size when stocked. The IDFG currently buys a portion of their rainbow trout eggs for catchable production from one commercial supplier, Trout Lodge. This company only supplies all-female eggs. If vulnerability to angling is less for female rainbow trout as it was for juvenile female coho, use of all-female populations for the catchable program could decrease return to creel rates.

MANAGEMENT GOAL

1. To maintain or improve angler success rates for put-and-take rainbow trout in streams.

OBJECTIVES

1. To determine if Hayspur broodstock selected for their angling vulnerability produce offspring that return to angler creels 25% more often than normal hatchery trout.
2. To determine if vulnerability to angling of male rainbow trout exceeds that of females by 25% within the Hayspur broodstock.
3. To compare relative return across 2001 and 2002 stocking locations and determine which stocking locations meet the state's stocking criteria of 40% return by number.

DESCRIPTION OF STUDY AREA

Sixteen study sites were stocked in 2001, including 10 stream or river segments, three reservoirs, and three ponds. Study sites were located in the Big Wood, Big Lost, and upper Snake River watersheds (Figure 1). An additional 16 study sites were stocked in 2002, including

15 different stream or river segments with two sites on the North Fork Payette River. Two dams separated study sites on the North Fork Payette River. The majority of sites stocked in 2002 were within the Payette, Boise, and Weiser river watersheds, but individual sites were also located in the Portneuf River and Rock Creek.



METHODS

A series of fishing trials was conducted in 1999 on one-year-old rainbow trout designated to become replacement broodstock at Hayspur Fish Hatchery. Prior to fishing trials, passive integrated transponder (PIT) tags were injected into the abdominal cavity of all fish. Fish caught three or more times during the trials were uniquely marked in case of tag loss and retained for establishing the experimental group. For additional description of fishing trials and broodstock selection procedures, see Teuscher (1999).

During November 1999 and 2001, male and female rainbow trout that were captured more than three times in the fishing trials were spawned, and their progeny were used as the experimental group in this study, hereafter referred to as vulnerable. Control or normal groups were also created during the same time periods from normal Hayspur strain rainbow trout. Normal and vulnerable eggs were transported from Hayspur to Ashton and Nampa fish hatcheries. Eggs and fry were reared separately until length distributions and condition factors became relatively equal. The vulnerable group was adipose-clipped and combined with the normal group into one outside raceway until the time of stocking. I assumed that removal of the adipose fin had no effect on survival or catchability (Heimer et al. 1985).

During the initial egg take in 1999, a random sample of replacement brood fish was sexed as fish were spawned to assess whether vulnerability to angling differed between sexes. Sex was determined visually by examining gonadal products after applying light pressure to the abdomen. I tested the null hypothesis that capture frequencies were equal for male and female rainbow trout by comparing sex to capture data. The observed and expected capture frequencies by sex were compared with the chi-square statistic using a 2 X 4 contingency table (Zar 1996). Capture frequencies were grouped into four categories: 0, 1, 2, and 3 or more. Expected frequencies were calculated by multiplying the proportion of each sex by the number of fish determined to be in each capture category.

Equal numbers of fish from the two groups were jaw-tagged at Ashton Fish Hatchery during May 2001 and at Nampa Fish Hatchery during May and June 2002. One hundred trout from each group were measured for length and weight before tagging. During 2001, study fish in the rearing raceway were crowded, and 6,400 catchables were randomly removed, anesthetized, jaw tagged, and held in holding pens for 0.5 to 20 hours. Each # 8 Monel jaw tag was labeled "RTN IFG" and numbered. Jaw tag numbers identified group and stocking site. The PVC holding pens were 1.2 x 1.2 x 2.4 m (width x height x length) and were lined with 6 mm plastic hardware cloth. Immediate tag loss due to shedding or mortality was evaluated by examining raceway bottoms below the holding pens just before transport. Shed tags were reapplied to replacement trout, if observed. The same methods as above were used for the replicate trial conducted in 2002, except that the number of fish was increased to 9,600 catchables.

Approximately equal numbers of vulnerable and normal catchables were stocked in study waters from May 30 to June 6, 2001 and from May 15 to June 19, 2002. At streams and rivers, fish were dipnetted from the transport tank and released at several areas to encourage dispersal of tagged fish throughout the stocked stream reach. At ponds and reservoirs, fish were stocked through a discharge tube at one location, usually a boat ramp.

Reward incentives, press releases, personal contacts, and signs were used to increase awareness of the project and encourage angler tag reporting in returning tags. Anglers that

returned tags were entered in site-specific drawings where a single winner was awarded \$50. Newspaper, radio, and television were used to disseminate information regarding the location of the study waters, the reward incentive, and the project goal. Blaze-orange signs with information pertinent to the drawing were posted at two to eight locations near access points in all waters immediately after stocking. Additionally, data slips with the tag return instructions were affixed to each sign to assist anglers in the tag return process. Jaw tag data were collected by mail, telephone, and field contacts by IDFG personnel. Tag number, angler address, capture location, and catch date were entered and compiled in a database.

Tag returns were compiled by group and study site. All tags returned before December 31 were considered first year returns. Tags returned from January 1 through December 31 of the year following stocking were considered second year returns. Additionally, the number of days from stocking to harvest was determined for each return. Mean values and 95% confidence intervals were calculated for the length of each group prior to stocking as well as for the return rate and time to harvest by group for each stocking location (Brown and Austen 1996). Paired *t*-tests were used to test the null hypothesis that there were 1) no differences in the number of normal and vulnerable catchable tag returns, and 2) no differences in time to harvest (d) for normal and vulnerable catchables for each stocking year separately (Dillon et al. 2000).

Although estimates of overall tagged trout return rates (i.e., exploitation) were not primary goals of this study, such results are always of interest to fisheries managers. Consequently, total adjusted return-to-creel rate estimates were calculated for the 2001 plant, and adjusted first-year return rate estimates for the 2002 plants. The adjusted return to creel rate was calculated by dividing the observed tag return rate by an estimated tag reporting rate. Since the true rate is unknown, I corrected observed tag returns for each stocking location by a range of values likely to encompass the true tag reporting rate (Rieman 1987). These reporting rates were 0.3, 0.36, and 0.5. The lower rate, 0.3, would represent a low response rate from anglers who harvested tagged fish. The middle value, 0.36, is the mean tag reporting rate of several duck banding studies that used standard non-reward tags (Table 1). The upper value, 0.5, would represent a high response rate from anglers who harvested tagged fish and is my best estimate of the true tag reporting rate for this study in the absence of site-specific data. This assumption is based upon the likelihood that anglers returned \$50 lottery tags at a greater rate than duck hunters returned standard non-reward tags.

Table 1. Band or tag reporting rates from previous studies. Estimated tag return reporting rates (compliance) are listed for standard (non-reward) tags.

Reference	Species	Incentive (\$)	Estimated compliance (%)
Standard tag or band			
Nichols et al. 1991	Duck	None	32
Nichols et al. 1991—adjusted for bias	Duck	None	26
Henry and Burnham 1976	Duck	None	38
Nichols et al. 1995	Duck	None	38
Conroy and Blandin 1984	Duck	None	43
Reeves 1979	Dove	None	38
Average			36

RESULTS

Vulnerability to Angling by Sex

On October 19, 1999, the sex of 1,250 replacement brood fish was identified during the initial egg take. A total of 488 males and 762 females were identified, yielding a sex ratio of 39% male and 61% female (Table 2). Observed and expected capture frequencies were within two individuals or less for the zero and three or more capture categories. For the one-capture category, eight more males were caught than expected. For the two-capture category, seven more females were captured than expected. Although there were a few departures from expected frequencies, there was no statistical difference in the frequencies at which males and females were caught ($\chi^2 = 1.81$, $p = 0.62$, $df = 3$).

Table 2. Observed and expected capture frequencies for male and female Hayspur strain rainbow trout. There was no statistical difference in catchability between males and females ($\chi^2 = 1.81$, $p = 0.62$, $df = 3$).

Sex	Capture Frequency				Total (Observed)
	0	1	2	3+	
Male (Observed)	86.00	93.00	222.00	87.00	488
Male (Expected)	87.06	84.72	229.95	86.28	488
Female (Observed)	137.00	124.00	367.00	134.00	762
Female (Expected)	135.94	132.28	359.05	134.72	762
Total (Observed)	223	217	589	221	1250

2001 Stocking

At the time of stocking, mean lengths of the test groups were not statistically different based on overlapping confidence intervals. Mean lengths of the vulnerable and normal groups were 244.7 mm (± 4.3 mm) and 242.8 mm (± 5.4 mm), respectively.

No consistent differences in return rates were observed between the two test groups across 2001 stocking locations. A total of 414 tags were returned from the vulnerable group and 384 from the normal group over both years (Table 3). The number of tags returned from the vulnerable group was higher at 10 of 16 locations, whereas the number of tags returned from the normal group was higher at five locations. The number of returns was equal for the Henrys Fork at Mack's Inn. Relative tag returns between the test groups were within three tags or less at six locations and within five tags or less at nine locations. First year return rate for the vulnerable group ranged from 6.5% to 26.0% and averaged $12.7 \pm 3.5\%$. First year return rate for the normal group ranged from 3.0% to 30.0% and averaged $11.7 \pm 3.8\%$. First year return rates between groups were not statistically different (paired t -test, $p = 0.30$, $df = 15$).

Table 3. Stocking location, number of tagged fish stocked, number of tags returned by anglers, and return rate from each of 16 locations stocked in 2001 with catchable rainbow trout produced from parents that were highly susceptible to angling (Vulnerables) and Hayspur strain broodstock (Normals). Stocking locations are listed in order of total returns.

Stocking Locations for 2001	Date	Vulnerables					Normals				
		# Stock	1st Year Returns	2nd Year Returns	1st Year Return Rate (%)	2nd Year Return Rate (%)	# Stock	1st Year Returns	2nd Year Returns	1st Year Return Rate (%)	2nd Year Return Rate (%)
Ashton Reservoir	05/30/01	200	51	0	25.5	0.0	200	60	0	30.0	0.0
Warm River	05/30/01	200	52	0	26.0	0.0	200	47	0	23.5	0.0
Big Lost River	06/01/01	200	50	0	25.0	0.0	200	28	0	14.0	0.0
Roberts Gravel Pond	06/04/01	200	30	1	15.0	0.5	200	41	1	20.5	0.5
North Fork Big Wood River	06/05/01	200	29	0	14.5	0.0	200	31	0	15.5	0.0
Birch Creek	06/04/01	199	25	0	12.6	0.0	200	23	2	11.5	1.0
Trail Creek	06/05/01	200	27	0	13.5	0.0	200	23	0	11.5	0.0
Henrys Fork at Mack's Inn	05/30/01	199	24	0	12.1	0.0	200	24	0	12.0	0.0
Snake River at Idaho Falls	05/31/01	200	21	3	10.5	1.5	200	15	5	7.5	2.5
Mackay Reservoir	06/01/01	200	15	0	7.5	0.0	200	16	1	8.0	0.5
East Fork Big Lost River	06/05/01	200	17	0	8.5	0.0	199	14	0	7.0	0.0
Gem State Reservoir	05/31/01	199	13	3	6.5	1.5	199	12	1	6.0	0.5
Buffalo River	05/30/01	200	11	0	5.5	0.0	199	17	0	8.5	0.0
Harriman Fish Pond	05/30/01	199	16	0	8.0	0.0	199	9	0	4.5	0.0
West Fork Big Lost River	06/05/01	200	13	0	6.5	0.0	198	8	0	4.0	0.0
Sand Creek Pond 3	05/30/01	200	13	0	6.5	0.0	199	6	0	3.0	0.0
Total number or mean rate		3196	407	7	12.7	0.2	3193	374	10	11.7	0.3

Very few tags were returned from the second fishing season after stocking. Of the 798 tags returned, only 17 tags (2%) were second year returns (Table 3). These returns were comprised of seven tags from vulnerable fish and 10 tags from normal fish. The majority of second year returns ($n = 12$) came from two reservoir stocking locations, the Snake River in Idaho Falls and Gem State Reservoir. Tags were returned in successive years from only five stocking locations.

The vulnerable group tended to return to the creel more quickly during 2001 (Figure 2), but this disparity was not statistically different (paired t -test, $p = 0.77$, $df = 15$). The mean time to harvest was 46.4 ± 9.8 d for the vulnerable group and 50.6 ± 10.7 d for the normal group. Over 50% of the tags returned were from fish caught within 50 days of stocking, and over 85% were from fish caught within 100 days.

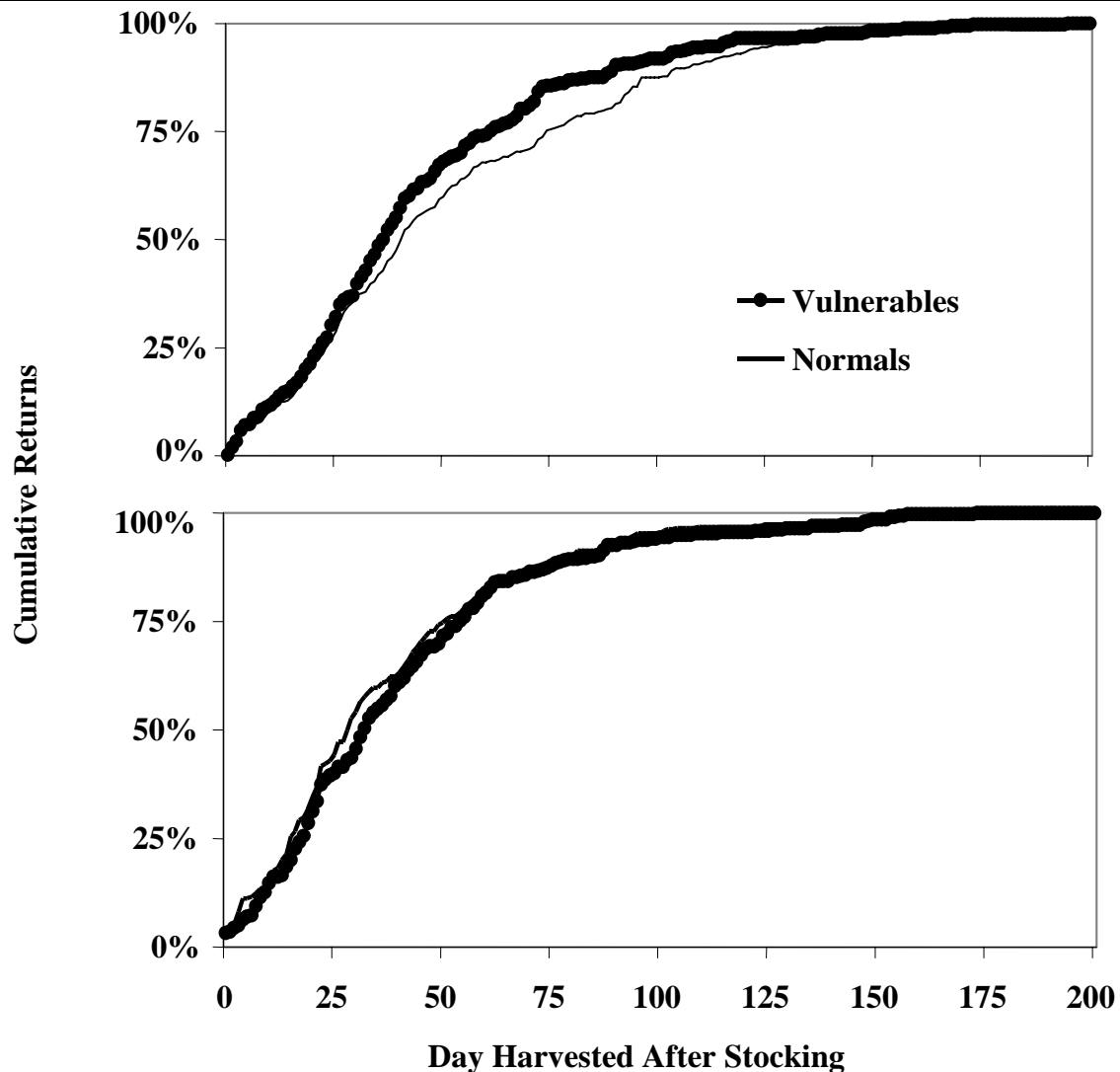


Figure 2. Cumulative first-year return to creel over time for vulnerable and normal hatchery rainbow trout that were stocked in 2001 (top) and 2002 (bottom). Time to harvest was combined by test group for all 16 stocking locations.

Total tag returns collected during 2001 and 2002 from jaw tagged rainbow trout stocked in 2001 varied widely across stocking locations. Out of 6,389 fish that were tagged and stocked during 2001, 798 tags were returned, yielding a total return rate of $12.5 \pm 3.7\%$ (Table 4). A more than five-fold difference in total returns existed between the locations with the highest (Ashton Reservoir; $n = 111$) and lowest (Sand Creek Pond #3; $n = 19$) returns. Assuming tag reporting was slightly higher (50%) than the mean of past studies (36%; Table 1), adjusted return to creel rates averaged $25.0 \pm 6.9\%$ (Table 4). Rates were highest for Ashton Reservoir and Warm River at 55.5% and 49.5%, respectively. Adjusted return-to-creel rates were less than 40% in 14 of the 16 stocking locations.

Table 4. Number of tagged rainbow trout stocked, number of returns by anglers, return rate, and adjusted return rate for locations stocked during 2001. The adjusted rate was calculated by dividing return rate by a range of tag reporting rate estimates (see text for citations). Results from 2001 locations include first and second year returns.

Stocking Location in 2001	Totals			Adjusted Return Rate (%)		
	# Stock	# Return	Return Rate (%)	C = 0.3	C = 0.36	C = 0.5*
Ashton Reservoir	400	111	27.8	92.5	77.1	55.5
Warm River	400	99	24.8	82.5	68.7	49.5
Big Lost River	400	78	19.5	65.0	54.2	39.0
Roberts Gravel Pond	400	73	18.3	60.8	50.7	36.5
North Fork Big Wood River	400	60	15.0	50.0	41.7	30.0
Birch Creek	399	50	12.5	41.8	34.8	25.1
Trail Creek	400	50	12.5	41.7	34.7	25.0
Henrys Fork at Mack's Inn	399	48	12.0	40.1	33.4	24.1
Snake River at Idaho Falls	400	44	11.0	36.7	30.6	22.0
Mackay Reservoir	400	32	8.0	26.7	22.2	16.0
East Fork Big Lost River	399	31	7.8	25.9	21.6	15.5
Gem State Reservoir	398	29	7.3	24.3	20.2	14.6
Buffalo River	399	28	7.0	23.4	19.5	14.0
Harriman Fish Pond	398	25	6.3	20.9	17.4	12.6
West Fork Big Lost River	398	21	5.3	17.6	14.7	10.6
Sand Creek Pond 3	399	19	4.8	15.9	13.2	9.5
Total number or mean rate	6389	798	12.5	41.6	34.7	25.0

*assumed tag reporting rate

2002 Stocking

At the time of stocking, mean lengths of the test groups were not statistically different based on overlapping confidence limits. Mean length of the vulnerable and normal groups was 247.0 ± 5.3 mm and 248.2 ± 3.6 mm, respectively.

High variability between stocking locations and lack of statistical difference in returns between the test groups were similar to the pattern observed for fish stocked in 2001. Three hundred forty-seven tags were returned from the vulnerable group and 353 from the normal

group (Table 5). First year return rate for the vulnerable group, unadjusted for tag reporting, ranged from 0.7% to 16.3% and averaged $7.2 \pm 2.5\%$. Unadjusted first year return rate for the normal group ranged from 0.0% to 19.1% and averaged $7.4 \pm 2.7\%$. First year return rates between groups were not statistically different (paired *t*-test, $p = 0.80$, $df = 15$).

In contrast to the results of 2001 stocking, the normal group tended to return to the creel more quickly (Figure 2), but the small disparity observed was not statistically significant (paired *t*-test, $p = 0.45$, $df = 15$). The mean time to harvest was 36.0 ± 8.0 d for the normal group and 38.7 ± 7.3 d for the vulnerable group. Over 50% of the tags returned were from fish caught within 32 days after stocking, and over 95% were from fish caught within 100 days.

First year tag returns from fish stocked in 2002 were on average about 5% lower than for locations stocked in 2001. Total first year return rate (both groups combined) unadjusted for tag reporting ranged from 0.3% to 17.9% (Table 5). Out of 9,593 fish that were tagged and stocked, 704 tags were returned (Table 6). Eleven of the 16 stocking locations had total first year adjusted return rates of less than 10%, whereas only one of the 16 stocking locations exceeded 15% total first year return rate. Assuming tag reporting rate equaled 50%, none of the 16 stocking locations stocked in 2002 had adjusted return rates that met or exceeded the IDFG Fish Management Goal of 40% by number (IDFG 2001). Six out of 16 stocking locations in 2002 had adjusted return rates of 10% or less.

Table 5. Stocking location, number of tagged fish stocked, number of tags returned by anglers, and return rate from each of 16 locations stocked in 2002 with catchable rainbow trout produced from parents that were highly susceptible to angling (Vulnerables) and Hayspur strain broodstock (Normals). Stocking locations are listed in order of total returns.

Stocking Locations for 2002	Date	Vulnerables					Normals				
		#	1st Year	2nd	1st Year	2nd Year	#	1st Year	2nd	1st Year	2nd Year
		Stock	Returns	Year	Return	Return	Stock	Returns	Year	Return	Return
				Returns	Rate (%)	Rate (%)			Returns	Rate (%)	Rate (%)
Boise River-Town Section	06/18/02	300	49	—	16.3	—	299	57	—	19.1	—
Warm Springs Creek	06/20/02	300	38	—	12.7	—	300	38	—	12.7	—
Rock Creek	05/15/02	300	28	—	9.3	—	300	39	—	13.0	—
North Fork Boise River	06/19/02	299	35	—	11.7	—	299	30	—	10.0	—
Middle Fork Weiser River	06/18/02	300	33	—	11.0	—	300	28	—	9.3	—
Portneuf River	05/17/02	300	31	—	10.3	—	300	28	—	9.3	—
NF Payette River-55 Crossing	05/16/02	297	29	—	9.8	—	299	25	—	8.4	—
Mores Creek	05/23/02	300	22	—	7.3	—	300	28	—	9.3	—
MF Boise River-above Atlanta Dam	06/19/02	300	25	—	8.3	—	300	15	—	5.0	—
NF Lake Fork Creek	06/27/02	300	15	—	5.0	—	300	17	—	5.7	—
Little Smoky Creek	06/21/02	300	14	—	4.7	—	300	12	—	4.0	—
Silver Creek	05/21/02	300	7	—	2.3	—	300	16	—	5.3	—
Weiser River	05/24/02	300	9	—	3.0	—	300	10	—	3.3	—
Crooked River	05/23/02	300	9	—	3.0	—	300	7	—	2.3	—
NF Payette River-above Payette L.	06/27/02	300	1	—	0.3	—	300	3	—	1.0	—
Grimes Creek	05/21/02	300	2	—	0.7	—	300	0	—	0.0	—
Total number or mean rate		4796	347	—	7.2	—	4797	353	—	7.4	—

Table 6. Number of tagged rainbow trout stocked, number of returns by anglers, return rate, and adjusted return rate for locations stocked during 2002. The adjusted rate was calculated by dividing return rate by a range of tag reporting rate estimates. (See text for citations.) Results from 2002 locations include 1st year returns only.

Stocking Location in 2002	Totals			Adjusted Return Rate (%)		
	# Stock	# Return	Return Rate (%)	C = 0.3	C = 0.36	C = 0.5*
Boise River-Town Section	599	107	17.9	59.5	49.6	35.7
Warm Springs Creek	600	76	12.7	42.2	35.2	25.3
Rock Creek	600	67	11.2	37.2	31.0	22.3
North Fork Boise River	598	66	11.0	36.8	30.7	22.1
Middle Fork Weiser River	600	61	10.2	33.9	28.2	20.3
Portneuf River	600	59	9.8	32.8	27.3	19.7
NF Payette River-55 Crossing	596	56	9.4	31.3	26.1	18.8
Mores Creek	600	50	8.3	27.8	23.1	16.7
MF Boise River-above Atlanta Dam	600	40	6.7	22.2	18.5	13.3
NF Lake Fork Creek	600	32	5.3	17.8	14.8	10.7
Little Smoky Creek	600	26	4.3	14.4	12.0	8.7
Silver Creek	600	23	3.8	12.8	10.6	7.7
Weiser River	600	19	3.2	10.6	8.8	6.3
Crooked River	600	16	2.7	8.9	7.4	5.3
NF Payette River-above Payette L.	600	4	0.7	2.2	1.9	1.3
Grimes Creek	600	2	0.3	1.1	0.9	0.7
Total number or mean rate	9593	704	7.3	24.5	20.4	14.7

*Assumed tag reporting rate

DISCUSSION

The tendency for the vulnerable group to produce a very slight overall increase in tag returns and to return more quickly than the normal group from 2001 stocking locations was not substantiated by returns from 2002 stocking locations. The opposite pattern was observed in 2002, with the normal group returning in slightly greater numbers and more quickly than the vulnerable group. The small, insignificant differences observed between test groups in return rates and times to harvest as well as the lack of a consistent pattern across years suggest that no performance benefit was achieved through selective breeding over one generation. Certainly the small observed differences were not important from a management perspective. This result contradicts conclusions drawn by other researchers who have artificially selected for specific behavioral traits in other species. In fishing trials, Garrett (1993) showed varying levels of vulnerability to angling in largemouth bass. Selective breeding of the highly vulnerable fish produced progeny that were more likely to be caught, especially when brood fish had been caught two, three, or four times. Gerlai and Csányi (1994) were able to increase and decrease a behavioral movement pattern in paradise fish *Macropodus opercularis* by selecting parents who had high and low expressions of this behavior. The movement pattern was inherited strongly by the F₁ generation, and selection in subsequent generations did not change the behavior substantially. David Phillips (Illinois Natural History Survey, unpublished data) has demonstrated that largemouth bass angling vulnerability is quite heritable, and that selective breeding for more catchable fish can be demonstrated with F₁ and F₂ crosses.

There are several possible reasons for our contradictory results. The previous studies examined the difference between low and high expressions of a particular behavior. Due to space and a desire to run a test at a viable production scale, I sought only to compare the difference between the normal Hayspur brood fish progeny and those whose parents showed high vulnerability to angling. It is possible that selection for more vulnerable catchables does occur, but the effect might not be large for test fish when compared to our normal or average fish. Furthermore, the brood fish for previous studies were selected from wild or naturalized populations, where individuals likely possess more genetic diversity than the Hayspur broodstock. Although a difference in vulnerability to angling was shown for Hayspur brood fish (Teuscher 1999), trials were conducted while fish were strictly confined (raceways) and subjected to intense fishing effort (94 h) with a limited number of fly patterns (3) and lures (3). In contrast, Garrett (1993) observed differences in vulnerability to angling with 40 hours of fishing effort, and fishing trials were conducted over a larger enclosure (0.25 hectare pond). Thus, several differences in experimental design could explain the disparate results of the present effort compared to other studies. Nonetheless, based on our results from the 2001 and 2002 stocking years, I believe that selective breeding for angling vulnerability will not increase returns.

First year return rate of jaw tags may show considerable variation across years. Dillon et al. (2000) examined the relative performance of diploid and triploid rainbow trout by stocking 16 Idaho streams during 1997. No difference was found in relative performance, so the results are comparable with the present study. These two studies had similar designs, including 12 of the same stocking sites, the use of angler returned jaw tags to compare relative performance, and test groups consisting of Hayspur-strain rainbow trout. Over this five to six year period, first year return rates had declined in all common stocking locations. The decline in return rate averaged 10.7% with a maximum of 30% in Birch Creek. The consistency and magnitude of decline is concerning and may have been caused by decreased survival, effort, harvest, or tag reporting.

The current Idaho fish management plan sets the harvest goal (exploitation) for stocked catchable trout at 40%. I used a point tag reporting rate estimate (50%) based on past studies and the assumption that \$50 lottery tags have at least some perceived value. The adjusted return to creel rates using a 50% tag reporting rates indicated that only a small portion, 2 of 16, of the 2001 stocking locations met the IDFG stocking goal of 40% return by number. Five stocking locations did not reach 15% by number, including Gem State Reservoir, Buffalo River, Harriman Fish Pond, West Fork Big Lost River, and Sand Creek Pond 3. Few of the 2002 stocking locations are likely to reach the stocking goal, although only first year returns are available. Eight stocking locations had adjusted return to creel estimates of less than 15%, whereas only one exceeded 35%.

Miranda et al. (2002) cautioned against using angler tag returns to estimate exploitation, as many basic marking and recapture assumptions may be violated (Ricker 1975). Miranda et al. (2002) speculated that such factors as post-release mortality caused by tagging stress, emigration, differential catchability of tagged and untagged fish, tag loss, and imprecise tag reporting rate estimates yield wide confidence intervals around exploitation rates. It is unlikely that tagging mortality, emigration, or tag loss after release significantly affected this study. Tagged fish were often held in net pens for up to 20 hours after tagging, and few mortalities were observed (less than five fish combined over both tagging years). Jaw tags have been shown to be a persistent mark. Retention of # 8 Monel jaw tags, the same tags used for this study, on rainbow trout was 99% at six months after tagging and 97% at 14 months (Stauffer and Hansen 1969). For the present study, test fish were stocked after high water had receded and at temperatures above 10°C to reduce the probability of emigration. Returns from jaw

tagged rainbow trout stocked in the upper Salmon River near Challis indicated that fish remained near stocking sites until after their first winter and gradually dispersed thereafter (Bjornn and Mallet 1964). In the upper Salmon River, over 90% of first year returns were caught within two miles of the stocking site, and over 90% of second year returns were caught within five miles.

However, differential catchability of tagged and untagged fish may have biased adjusted return-to-creel rates. Jaw tags may alter the catchability or survival of stocked rainbow trout. Using two different stocking methods, returns of fin-clipped rainbow trout were 1.2 and 1.5 times higher than for jaw tagged rainbow trout in the Pigeon River, Michigan (Cooper 1952). Therefore, the use of jaw tags in the present study could have caused underestimated adjusted return to creel rates.

Probably the greatest source of uncertainty in estimating exploitation rates through tag returns is the difficulty and high cost of accurately estimating tag reporting rates. To reduce the uncertainty in the absence of my own data, I calculated the mean tag reporting rate from past published studies ($n = 6$), and the range was set conservatively. This should provide reasonable estimates of exploitation; however, none of the existing studies cited have estimated tag reporting rates for anglers, only hunters. Additionally, these past studies used non-reward tags (i.e., no value). The tags used during the present study offered a chance to win \$50 and, therefore, had at least some perceived value. Increased perceived value of the tag would likely lead to higher tag reporting. However, it is impossible to estimate the level of error resulting from the use of lottery tags and reporting rates calculated from hunting studies. For this reason, in the future, this project should conduct studies to quantify angler tag reporting rates. Such studies would include 1) construction of tag response curves as included in a duck banding study (Nichols et al. 1991) and 2) an assessment of jaw tag effects on individual fish catchability.

Due to the possible decreased catchability associated with jaw tags, uncertainty of tag reporting rates, and possible declining interest in returning tags, it is most appropriate to interpret my adjusted return-to-creel rates cautiously. These data would be best used to compare relative returns across stocking locations. This would allow fisheries managers to prioritize stocking to higher return-to-creel locations when shortages of catchables occur.

RECOMMENDATIONS

1. Discontinue collecting tag returns from 2002 stocking locations in 2003. Due to low first year tag returns and no reservoir stocking locations, it is probable that second year tag returns from 2002 stocking location will be very low.
2. Do not use selective breeding of the Hayspur broodstock as a tool for increasing return to creel.
3. Estimate tag reporting rates and differential catchability of jaw tagged and untagged rainbow trout in Idaho to allow more precise estimates of adjusted return to creel rates.

ACKNOWLEDGEMENTS

I would like to thank Dennis Lee, Mike Colvin, and Sarah Nelson for their work on all aspects of this project, especially for maintaining the tag return database and the reward signs. Rick Alsager, Dan Baker, Bob Turik, Mel Sadecki, and Leon Martindale reared the test groups, provided information about specific stocking locations, and helped stock the test groups. Bob Esselman, Doug Young, and Russ Wood sorted, reared, and spawned the broodstocks. Steve Elle, Liz Mamer, Ken Felty, Travis Brown, Shane Claborn, and Eric Grohs helped tag and stock the test groups. Dan Schill, Dave Teuscher, and Doug Megargle have all participated in this project at various phases and provided advice.

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**ANNUAL PERFORMANCE REPORT
SUBPROJECT #2: STERILE TROUT EVALUATIONS**

State of: Idaho Grant No.: F-73-R-25 Fishery Research
Project No.: 4 Title: Hatchery Trout Evaluations
Subproject #2: Sterile Trout Investigations
Contract Period: July 1, 2002 to June 30, 2003

ABSTRACT

Increased growth, improved survival, and genetic protection of wild stocks have been suggested as possible benefits of stocking triploid (i.e., sterile) fish. I examined the relative growth and survival of triploid and diploid rainbow trout *Oncorhynchus mykiss* stocked in four high mountain lakes during 1999 and assessed grit retention for two groups of fish stocked in 16 high mountain lakes during 2001. Eight triploid and 34 diploid rainbow trout were collected from three of the four high mountain lakes during 2002. Combined with the catch statistics from 2001 (14 triploids and 20 diploids), the total catch of diploids was 2.5 times higher than for triploids over the two years of sampling. For all lakes combined, mean total length of the diploid group ($\bar{x} = 309 \pm 7$ mm; $n = 34$) was slightly longer than the triploid group ($\bar{x} = 302 \pm 23$ mm; $n = 8$), but there was no statistical difference. Similarly, mean weight of the diploid group ($\bar{x} = 301 \pm 18$ g; $n = 34$) was heavier than the triploid group ($\bar{x} = 286 \pm 64$ g; $n = 8$), but there was no statistical difference. For the test groups stocked during 2001, green dye was retained in 131 out of 139 diploid rainbow trout, or $94.2 \pm 5.5\%$, over a period of 393 days. Over the same time period, red dye was retained in 114 out of 130 triploid rainbow trout or $87.7 \pm 7.5\%$.

Additionally, methods for producing triploid brook trout *Salvelinus fontinalis*, rainbow by Yellowstone cutthroat trout hybrids *O. mykiss* X *O. clarki bouvieri*, kokanee *O. nerka*, and lake trout *S. namaycush* were tested. Three thermal treatments provided 100% induction rates in brook trout, and the highest survival rate (62.3%) among these treatments was provided by 29.4°C at 18 minutes after fertilization (MAF) for 7 min duration. The use of pressure to create triploid rainbow by Yellowstone cutthroat trout hybrids provided higher and more consistent induction rates than thermal treatments tested previously. The 69 megapascals at 40 MAF treatment provided mean survival and induction rates of 43 and 100%, respectively. For kokanee, survival of controls exceeded that of treatments by 14% or more. Kokanee induction rates approached or exceeded 90% for all but the two least intense treatments. The best combination of survival (48.7%) and induction rates (98.3%) was provided by shocking kokanee eggs at 27°C at 20 MAF for 20 min duration. No treatment was determined for producing high induction rates in lake trout. The highest induction rates (62.5%) were provided by 29.4°C at 18 MAF for 7 min duration treatment, but survival to eye up was only 39.6%, and substantial additional mortality occurred at hatch.

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INTRODUCTION

Triploid salmonids are functionally sterile, and the common assertion is that sterility provides a fisheries or aquaculture benefit (Benfey 1999). Triploid salmonids produced by temperature or pressure shock may suffer lower fertilization rates, increased mortality, or reduced growth from egg through initiation of feeding (Solar et al. 1984; Happe et al. 1988; Guo et al. 1990; Oliva-Teles and Kaushik 1990; Galbreath et al. 1994; McCarthy et al. 1996). Despite these early rearing disadvantages, triploid performance appears to improve with age. Several investigators reported enhanced hatchery performance in terms of growth and food conversion for age-1 and older triploids (Lincoln and Scott 1984; Bye and Lincoln 1986; Boulanger 1991; Habicht et al. 1994; Sheehan et al. 1999).

Unlike the breadth of previous work reported for triploid salmonids in an aquacultural setting, published literature on the performance of triploid salmonids in natural environments is sparse. Brock et al. (1994) and Simon et al. (1993) reported lower growth and survival for triploid rainbow trout *Oncorhynchus mykiss* compared to diploid controls. In contrast, triploid brook trout *Salvelinus fontinalis* and kokanee *O. nerka* demonstrated the potential for increased longevity in lake habitats (Parkinson and Tsumura 1988; Warrillow et al. 1997). Dillon et al. (2000) reported that stocking of mixed-sex triploid rainbow trout in 16 Idaho streams did not reduce return to creel for anglers compared to mixed-sex diploid fish. Lastly, Cotter et al. (2000) argued that stocking triploid Atlantic salmon *Salmo salar* reduced genetic impacts to wild populations, because fewer triploid fish returned to spawning habitats. These studies provide some background for evaluating the performance of triploid salmonids in natural environments. However, their limited scope, lack of replication, and contradicting results fail to fully address the performance of triploid salmonids stocked to benefit anglers.

The genetic conservation of wild populations is a management priority for the Idaho Department of Fish and Game (IDFG). The IDFG recently established a policy to stock only triploid rainbow trout in systems where reproduction between wild and hatchery fish was possible (IDFG 2001). Implementation of the above-noted policy has resulted in the widespread stocking of sterile rainbow trout in hundreds of Idaho high mountain lakes. In these lakes, temperature and oxygen levels may be low for much of the year, and it has been suggested that sterile fish may not perform well under these conditions (J. Johnston, Washington Department of Fish & Wildlife, personal communication). It is important to determine if stocking of triploid rainbow trout produces satisfactory fisheries in Idaho high lakes. If not, fisheries managers may need to adjust stocking strategies, rather than rely on historical stocking levels as is currently being done.

Induced sterility in other hatchery-produced salmonids including kokanee, cutthroat by rainbow hybrids *O. clarki bouvieri* X *O. mykiss*, as well as brook and lake trout *S. namaycush* could further reduce impacts of stocking on Idaho's native and wild fish populations. However, sterilization techniques for these species have yet to be developed in IDFG hatcheries. If efficient methods are developed, sterility may provide a method for controlling population levels of stocked lake trout in Bear Lake that have the potential to compete with or prey upon cutthroat trout (Kaeding et al. 1996). Another potential benefit of sterility is increased longevity through elimination of normal gonadal development and associated spawning mortality (Ihssen et al. 1990). Kokanee fisheries become marginal when individuals mature early and die before reaching a size desired by anglers (Rieman and Maiolie 1995). In British Columbia, sterile kokanee persisted through age-7, whereas only four diploid kokanee were captured after age-4 and none were captured after age-5 (Johnston et al. 1993). Increased kokanee longevity could

dramatically improve hatchery supported kokanee fisheries in Idaho by increasing the number of years kokanee are susceptible to anglers. As mentioned earlier, sterility also provides a level of genetic protection for wild stocks. This would be desirable for the management of Henrys Lake, as diploid hybrids have the potential to hybridize with the lake's Yellowstone cutthroat trout (Rohrer and Thorgaard 1986).

In this progress report, I compared preliminary survival and growth of triploid and diploid rainbow trout stocked as part of a pilot study in four central Idaho high mountain lakes. I also documented grit retention rates for a study on 16 additional waters that were stocked in 2001. In addition, I summarize efforts to produce triploid brook trout, rainbow X Yellowstone cutthroat trout hybrids, kokanee, and lake trout.

RESEARCH GOAL

1. To enhance hatchery-supported lake and reservoir fisheries, while reducing genetic risks to indigenous rainbow and cutthroat trout.

OBJECTIVES

1. To increase survival of rainbow trout in high mountain lakes by 25% by stocking all-female triploid fish, while maintaining growth rates equal to that of diploid rainbow trout. Assessments will include four high mountain lakes during 2001-2003 and an additional 16 lakes during 2004 and 2005.
2. To develop techniques for inducing triploidy in brook trout, rainbow X Yellowstone cutthroat trout hybrids, kokanee, and lake trout at high rates (95-100%), while maintaining adequate survival (not less than 75% of untreated fish).

METHODS

Performance of Sterile Trout in High Mountain Lakes

Pilot Study

McCall regional management personnel purchased normal (diploid) and pressure-treated (triploid) all-female Kamloops strain rainbow trout eggs from Trout Lodge commercial fish hatchery. Eggs were transported to McCall Fish Hatchery and incubated. Resultant fry were reared in 1 m tanks until they reached 50 mm and then transferred to raceways. Prior to stocking, the diploid and triploid groups were grit marked with green and red fluorescent dye, respectively. Fish were held for two weeks to monitor retention. Initial marking success was 94% for the triploid group (red) and 98% for the diploid group (green). Equal numbers of diploid and triploid fish were stocked into four lakes near McCall, Idaho with fixed-wing aircraft (Figure 3). On October 15, 1999, 500 diploid and 500 triploid fry were stocked into Maki, Golden, and Snowslide lakes, whereas 250 diploid and 250 triploid fry were stocked into Crystal Lake.

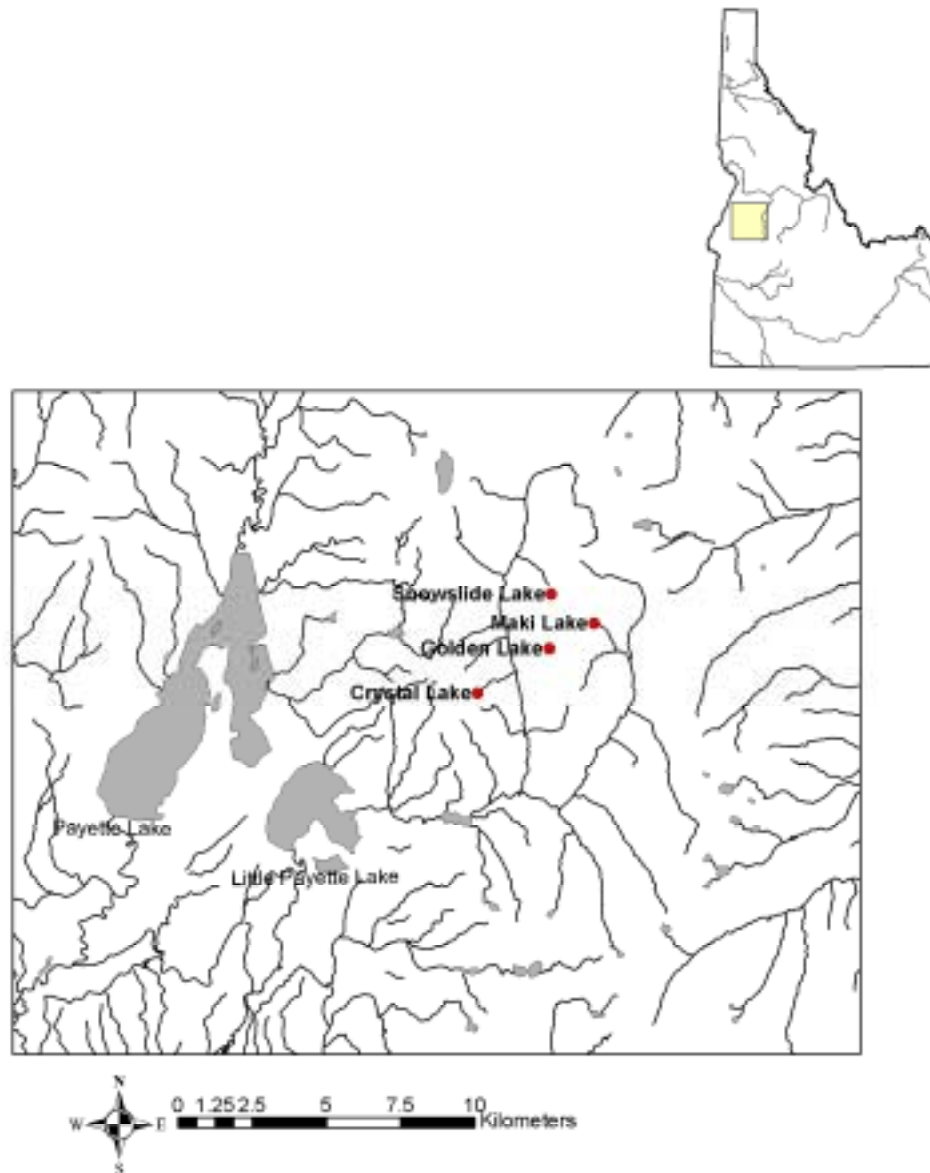


Figure 3. Location of four pilot study lakes stocked with diploid and triploid rainbow trout during 1999.

Lakes were surveyed with floating gillnets and angling from July 22 through July 31, 2002. The experimental gillnets used had 19, 25, 30, 33, 38, and 48 mm bar mesh panels and were 46 m long by 1.5 m deep. Typically, two gillnets were set in the early afternoon and pulled the following morning. While the nets fished, the two- or three-person field crew used spin- and fly-fishing gear to collect additional samples.

Captured fish were identified to species, measured in total length to the nearest millimeter, and weighed to the nearest gram. All rainbow trout were examined for grit mark presence under a portable fluorescent lantern (Model #UVL-4, UVP, Inc.). Examination for grit dye was conducted in the absence of light within a black plastic garbage bag.

Full-Scale Diploid vs. Triploid Assessment

To monitor grit retention of the test groups stocked in 2001 (Kozfkay and Megargle 2001), approximately 150 diploid and 150 triploid rainbow trout that possessed grit dye were moved to Eagle Fish Hatchery and held in separate rearing tanks. Test fish were initially held in 0.5 m tanks but were transferred to 1 and 2 m tanks as rearing densities increased. When the study was terminated in October 2002, all fish were anesthetized and examined for grit mark presence. Examination for grit dye was conducted by moving a portable fluorescent light along both sides of the fish within a black plastic garbage bag that blocked sunlight.

Production of Sterile Trout

Brook Trout

I conducted thermal shock experiments to induce triploidy in brook trout at Henrys Lake Fish Hatchery on October 28, 2002. Fertilized eggs for each replicate were produced by combining the gametes of three females with one male. (For experiment diagram see Figure 4.) Eggs were fertilized in a non-iodized saline solution (concentration of 7.4 g/L) to increase sperm viability and improve fertilization rates.

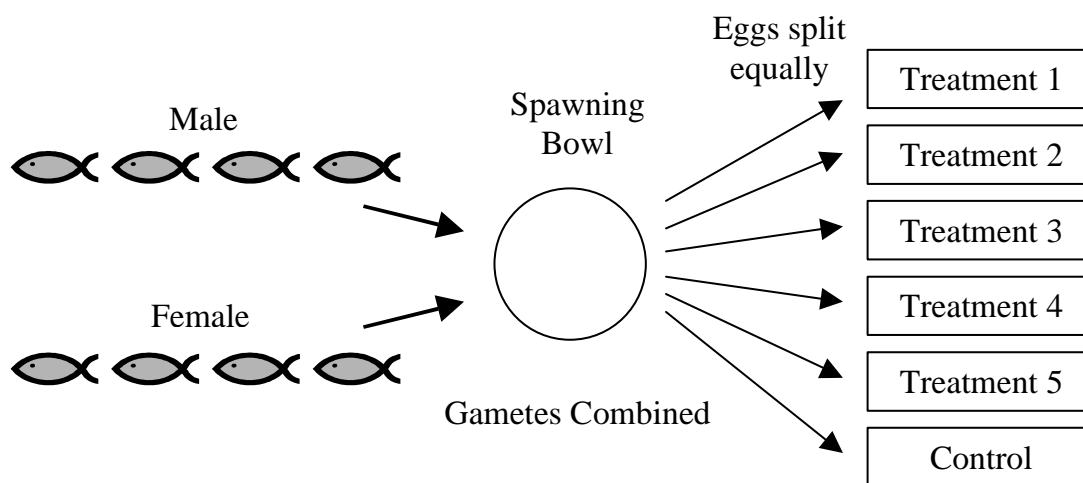


Figure 4. Theoretical diagram of one replicate in experiments designed to induce triploidy in brook trout, kokanee, and lake trout. For each additional replicate, another group of adults was used, and the process was repeated. The numbers of males, females, and treatments are examples only and in actuality varied for each experiment.

Eggs were placed in temporary containers until introduction into the heat bath at 10, 18, or 20 minutes after fertilization (MAF; Table 7). Approximately equal numbers of fertilized eggs (measured by displacement method) were split seven ways (six treatments and one control) and placed into Heath trays. Experimental treatment temperatures were 26, 27, 28, and 29.4°C, except the ambient hatchery water used for the control, which was 7.5°C. Treatment durations were 7, 10, or 20 minutes. After thermal treatment, eggs remained in Heath trays and were

placed in flow through vertical stacks. The process was repeated with three other females and one other male for each of the second and third replicates.

The interior dimensions of the hot water bath were 85.4 cm by 123.2 cm. The bath was filled to a depth of 10.8 cm, which yielded a volume of approximately 0.1 m³. Two heat pumps (PolyScience Inc., Model 210) and a recirculating pump (March Mfg. Inc., Model AC-3C-MD) were used to ensure that water temperatures remained stable and uniform.

All eggs were incubated, hatched, and reared at Henrys Lake Fish Hatchery. Eggs were enumerated at the eyed and hatch stages to determine survival. Blood samples were collected from each treatment group, stored in Alsever's solution, and shipped to Washington State University, where ploidy levels were determined with flow cytometry by Paul Wheeler and Brad Cunningham.

Table 7. Survival and triploid induction rates of brook trout from Henrys Lake during 2002 experiments that used various temperature, minutes after fertilization, and duration treatments.

Temp (°C)	MAF	Duration (Min)	Rep.	Survival-Eye Up (%)	Mean Survival Eye Up (%)	± SE	Induction Rate (%)	Mean Induction (%)	± SE
26	20	20	1	57.4	55.2	1.1	93.3	95.6	2.2
			2	54.0			93.3		
			3	54.2			100.0		
27	10	10	1	80.0	80.8	0.7	14.3	31.4	8.6
			2	80.3			40.0		
			3	82.3			40.0		
27	20	20	1	59.9	51.9	5.2	100.0	100.0	0.0
			2	53.4			100.0		
			3	42.2			100.0		
Control	20	20	1	90.3	88.8	0.7	0.0	0.0	0.0
			2	88.1			0.0		
			3	88.0			0.0		
28	10	10	1	56.1	50.3	2.9	100.0	100.0	0.0
			2	47.7			100.0		
			3	47.0			100.0		
28	20	20	1	0.3	2.2	1.0	NA	NA	NA
			2	2.6			NA		
			3	3.7			NA		
29.4	18	7	1	59.3	62.3	2.4	100.0	100.0	0.0
			2	60.6			100.0		
			3	67.0			100.0		

Henrys Lake Hybrids

I conducted pressure shock experiments to induce triploidy in rainbow x Yellowstone cutthroat trout hybrids at Henrys Lake Fish Hatchery on March 28, 2002 using fertilized eggs from normal hatchery production. Six females were fertilized with the milt from six Hayspur strain-males. After introduction of the milt and light stirring, eggs were rinsed with freshwater to initiate fertilization.

A small subsample of eggs was randomly selected from production eggs and set aside until either 300 or 400 thermal units had expired (Table 8). With the ambient water temperature of 7.5°C, eggs were introduced into the pressure apparatus at 40 and 53 MAF. The pressure level of experimental treatments was 62, 66, or 69 megapascals (Mpa; 9000-10000 Psi), except for the control, which was placed in the egg chamber without increasing the pressure. All pressure treatment durations were 5 minutes. After pressure treatment, eggs were placed in sealable plastic containers with fine-mesh screened tops and bottoms and stored in vertical flow through stacks. Survival and induction rates were estimated in the same manner as described in the brook trout section.

Table 8. Survival and triploid induction rates of Henrys Lake hybrids during 2002 experiments using various pressure and minutes after fertilization treatments.

Pressure (Mpa) (psi)	Rep.	Thermal Units	MAF	Survival Eye Up (%)	Induction Rate (%)	Mean Survival- Eye Up (%)	±SE	Mean Induction (%)	±SE
62 9000	1	300	40:00	55.0	5.0	61.9	3.9	10.0	7.6
	2			62.1	0.0				
	3			68.6	25.0				
Control	1	300	40:00	67.1	0.0	38.8	15.5	0.0	0.0
	2			13.6	0.0				
	3			35.7	0.0				
62 9000	1	400	53:20	55.0	0.0	73.8	9.5	11.8	9.2
	2			85.7	30.0				
	3			80.7	5.3				
66 9500	1	300	40:00	55.7	88.0	29.0	13.3	88.0	na
	2			16.4	na				
	3			15.0	na				
66 9500	1	400	53:20	48.6	94.4	36.9	17.7	86.4	11.0
	2			60.0	100.0				
	3			2.0	64.7				
69 10000	1	300	40:00	55.0	100.0	42.9	19.7	100.0	0.0
	2			69.3	100.0				
	3			4.3	100.0				
69 10000	1	400	53:20	65.0	95.0	53.1	8.0	91.6	2.0
	2			56.4	91.7				
	3			37.9	88.0				

Kokanee

I conducted thermal shock experiments to induce triploidy in kokanee at Deadwood Reservoir on August 30, 2002. Six to eight females were spawned with an equal number of males. Eggs were rinsed with freshwater to initiate fertilization and transported to a heated temporary shelter (wall tent) in spawning bowls.

Eggs were split seven ways (six treatments and one control) and placed in sealable plastic containers with fine mesh screened tops and bottoms (Table 9). Eggs and containers remained in buckets of river water until 10 or 20 MAF. Experimental treatment temperatures included 26, 27, 28, or 29°C, except that the ambient river temperature used for the control was 9°C. Treatment durations were 10 or 20 minutes. After thermal treatment, eggs were placed in a cooler of iced water and flown to Mackay Fish Hatchery with other normal production eggs. Kokanee were heat shocked with the same equipment as described in the brook trout section, and survival and induction rates were estimated in the same manner.

Table 9. Survival and triploid induction rates of kokanee from Deadwood Reservoir using various temperature, minutes after fertilization, and duration treatments conducted in 2002.

Temp (°C)	MAF (Min)	Duration (Min)	Rep.	Survival Eye Up (%)	Mean Survival Eye Up (%)	±SE	Induction Rate (%)	Mean Induction Rate (%)	± SE
26	20	20	1	66.6	61.6	3.6	70.6	46.3	12.5
			2	63.6			29.4		
			3	54.6			38.9		
27	10	10	1	41.5	44.3	1.9	40.0	27.9	7.0
			2	43.5			27.8		
			3	47.9			15.8		
27	20	20	1	54.4	48.7	2.9	100.0	98.3	1.7
			2	44.9			100.0		
			3	46.9			95.0		
Control	20	20	1	82.8	76.5	7.2	0.0	0.0	0.0
			2	62.1			0.0		
			3	84.6			0.0		
28	10	10	1	64.2	52.2	7.3	94.7	89.5	2.8
			2	39.0			85.0		
			3	53.4			88.9		
28	20	20	1	11.3	11.3	2.5	94.7	96.3	1.9
			2	15.6			100.0		
			3	6.9			94.1		
29	10	10	1	45.7	43.2	1.9	95.0	96.6	1.7
			2	39.6			94.7		
			3	44.5			100.0		

Lake Trout

Experiments to induce triploidy in lake trout were conducted in cooperation with the Utah Division of Wildlife Resources-Fisheries Experimental Station, Logan, Utah and the US Fish & Wildlife Service at Saratoga National Fish Hatchery, Saratoga, Wyoming on October 21, 2002. Five 8-year-old females were spawned with five males for each of the three replicates. Eggs were rinsed with freshwater to initiate fertilization.

Eggs remained in spawning bowls until just before introduction into the heat bath at 10, 15, or 18 MAF (Table 10). Approximately equal numbers of fertilized eggs (measured by displacement method) were split five ways (four treatments and one control) and placed into mesh bags. Experimental treatment temperatures were 28 or 29.4°C, except that the ambient hatchery water used for the control was 9.5°C. Treatment durations were 5 or 7 minutes. After removal from the bath, eggs were placed in Heath trays and placed in vertical flow through stacks in a random order. The process was repeated three times with different groups of eggs (replicates).

The heat shocking units consisted of individual 51 L insulated coolers. Coolers were fitted with inlet and outlet hoses and attached to one recirculating heat pump. Survival and induction rates were estimated in the same manner as described in the brook trout section.

Table 10. Survival and triploid induction rates of lake trout Saratoga National Fish Hatchery (Lewis Lake strain) during experiments conducted in 2002 using various temperature, minute after fertilization, and duration treatments.

(°C): MAF: Duration	Rep.	Egg- Eyed Egg (%)	Mean Egg-Eyed Egg (%)	± SE	Egg Hatch (%)	Mean Egg- Hatch (%)	± SE	Induction Rate (%)	Mean Induction (%)	± SE
Control	1	81.0	65.0	10.7	38.7	31.5	10.0	0.0	0.0	0.0
	2	69.4			44.0			0.0		
	3	44.6			11.8			0.0		
28.0: 10: 7	1	52.9	43.8	11.2	7.9	4.7	2.4	15.0	52.5	37.5
	2	57.0			6.0			90.0		
	3	21.4			0.0			NA		
28.0: 15: 7	1	59.8	49.9	10.4	12.0	7.1	3.6	30.0	45.0	15.0
	2	60.8			9.2			60.0		
	3	29.0			0.0			NA		
29.4: 18: 5	1	58.8	48.2	11.2	8.5	6.0	3.0	20.0	25.8	5.8
	2	60.0			9.4			31.6		
	3	25.8			0.0			NA		
29.4: 18: 7	1	45.8	39.6	10.6	7.2	4.0	2.1	40.0	62.5	22.5
	2	54.0			4.9			85.0		
	3	18.9			0.0			NA		

RESULTS

Performance of Sterile Trout in High Mountain Lakes

Pilot Study

No test fish were collected from Snowslide Lake during sampling on July 31, 2002, which may have been due to the presence of a wild brook trout population. Eight triploid and 34 diploid rainbow trout were collected from the other three lakes, most of which were sampled from Golden and Maki Lakes (Figure 5). In terms of total catch, gill net sets ($n = 29$) were more effective than hook and line sampling at capturing test fish ($n = 13$; Table 11). However, their relative effectiveness was unequal between the test groups. Catch per unit effort (CPUE) for triploids equaled 0.03 fish per hour for both methods. For diploids, hook and line sampling CPUE (0.35 fish per hour) exceeded that of gill net sets (0.09 fish per hour) by about 3.5 fold. Because of the relative difference in collection efficiencies among the two gear types, angling should be conducted as much as possible in future high lake evaluation of sterile fish.

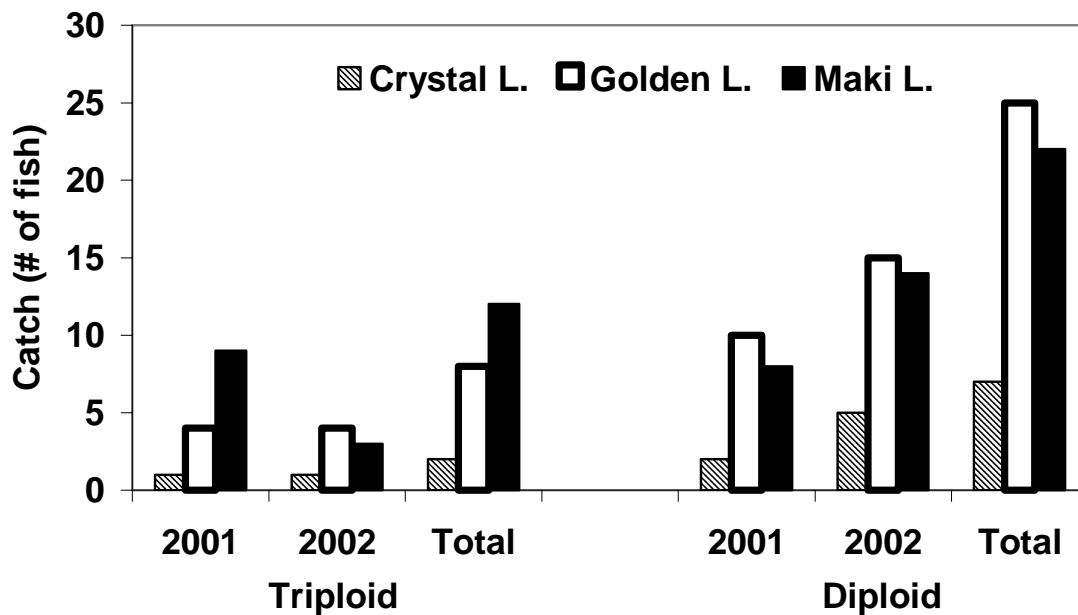


Figure 5. Yearly and total catch of diploid and triploid rainbow trout in pilot study lakes near McCall, Idaho.

Table 11. Catch, effort, and catch per unit effort (CPUE) for diploid and triploid rainbow trout surveys conducted on four high mountain lakes. Test fish were stocked as fry during October 1999.

Lake Name	Survey Date	Triploid						Diploid					
		Gill Net			Hook and Line			Gill Net			Hook and Line		
		Catch	Effort (h)	CPUE	Catch	Effort (h)	CPUE	Catch	Effort (h)	CPUE	Catch	Effort (h)	CPUE
Crystal	7/29/02	1	55	0.02	0	10	0.00	5	55	0.09	0	10	0.00
Golden	7/24/02	3	79	0.04	1	2	0.50	9	79	0.11	6	2	3.00
Maki	7/22/02	3	72	0.04	0	20	0.00	8	72	0.11	6	20	0.30
Snowslide	7/31/02	0	28	0.00	0	2	0.00	0	28	0.00	0	2	0.00
Totals		7	234	0.03	1	34	0.03	22	234	0.09	12	34	0.35

For all lakes combined, mean total length of the diploid group ($\bar{x} = 309 \pm 7$ mm; $n = 34$; Figure 6) was slightly longer than the triploid group ($\bar{x} = 302 \pm 23$ mm; $n = 8$), but not statistically different based on overlapping confidence intervals. In Golden Lake, mean length of triploids ($\bar{x} = 309 \pm 61$ mm; $n = 4$) exceeded that of diploids ($\bar{x} = 307 \pm 11$ mm; $n = 15$), but there was no statistical difference. This trend was reversed in Maki Lake, where mean length of diploids ($\bar{x} = 312 \pm 13$ mm; $n = 14$) exceeded that of triploids ($\bar{x} = 300 \pm 34$ mm; $n = 3$), but was not statistically different. Mean length of diploids ($\bar{x} = 306 \pm 18$ mm; $n = 5$) was also longer in Crystal Lake, but only one triploid was caught (283 mm).

Mean weight of the test groups followed a similar trend, with no statistical difference between groups within lakes. For all lakes combined, mean weight of the diploid group ($\bar{x} = 301 \pm 18$ g; $n = 34$; Figure 6) was heavier than the triploid group ($\bar{x} = 286 \pm 64$ g; $n = 8$). In Golden Lake, mean weight of triploids ($\bar{x} = 314 \pm 161$ g; $n = 4$) exceeded that of diploids ($\bar{x} = 304 \pm 29$ g; $n = 15$). This trend was reversed in Maki Lake, where mean weight of diploids ($\bar{x} = 306 \pm 35$ g; $n = 14$) exceeded that of triploids ($\bar{x} = 270 \pm 90$ g; $n = 3$). Mean weight of diploids ($\bar{x} = 282 \pm 37$ g; $n = 5$) was also longer in Crystal Lake, but only one triploid was caught (225 mm). Limited sample sizes clearly limit the overall utility of these pilot study results in terms of length and weight differences.

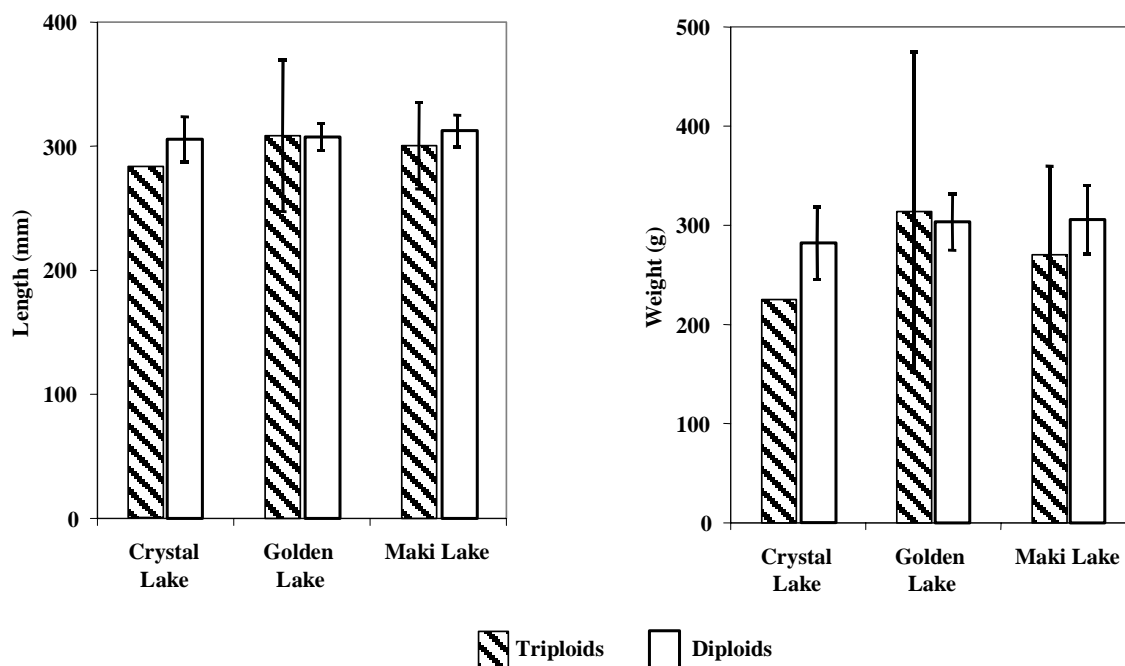


Figure 6. Mean length and weight of triploid and diploid rainbow trout that were stocked during October 1999 and sampled in June 2002. Errors bars indicate upper and lower 95% confidence limits.

Full-Scale Diploid vs. Triploid Assessment

Diploid and triploid rainbow trout initially identified as marked were held at Eagle Fish Hatchery for a period of 393 days from September 12, 2001 through October 9, 2002. At the time of transport, mean length of the diploid ($\bar{x} = 65 \pm 1.1$ mm) and triploid groups ($\bar{x} = 66 \pm 1.4$ mm) was equal. In addition, mean weight of the triploid group ($\bar{x} = 2.9 \pm 0.17$ g) was not statistically different from that of the diploid group ($\bar{x} = 2.6 \pm 0.13$ g). By the end of the period, mean length and weight of diploids had increased to 311 ± 7 mm and 480 ± 29 g, whereas triploids averaged 304 ± 15 mm and 475 ± 41 g. Green dye was retained in 131 out of 139 diploid rainbow trout remaining at the end of the experiment or $94.2 \pm 5.5\%$. Red dye was retained in 114 out of 130 triploid rainbow trout or $87.7 \pm 7.5\%$.

Production of Sterile Trout

Brook Trout

Survival of controls to eye-up was $88.8 \pm 0.7\%$ (Table 7). Survival for the 27°C: 10 MAF: 10 minute duration treatment ($80.8 \pm 0.7\%$) was only slightly less than that of controls. For the three treatment temperatures (26, 27, & 28°C) that were conducted at 20 MAF and 20 min duration, survival decreased as temperature increased, with a sharp decline in survival

occurring at 28°C. Within the same temperature, survival was higher for the treatments that were conducted at fewer MAF and for shorter duration.

Induction rates were 95% or greater for four of the five treatments tested (Table 7). No blood samples were collected from the 28°C: 20 MAF: 20 min duration treatment, because mean survival was below 3%. Three treatments had mean induction rates of 100%, including 27°C: 20 MAF: 20 min, 28°C: 10 MAF: 10 min duration, and 29.4°C: 18 MAF: 7 min duration recipes.

Henry's Lake Hybrids

Survival of controls equaled $38.8 \pm 15.5\%$ (Table 8). Survival of eggs to eye up was higher for pressure treatments than controls in four of six cases. The highest survival was seen in the two 62 Mpa treatments (62% and 74%), and variability was relatively low between replicates within these treatments. Survival was lower and more variable in the 66 and 69 Mpa treatments. Across all three pressure levels, survival was higher when eggs were shocked at 400 than at 300 thermal units.

Induction rates for hybrids increased as pressure levels increased. The lowest induction rates occurred at the 62 Mpa treatments and the highest at the 69 Mpa treatments (Table 8). Induction rates were very poor for the 62 Mpa treatments (<12%). For the 66 and 69 Mpa treatments, induction rates decreased at higher thermal units. Based on a 100% induction rate and survival to eye-up rates slightly greater than controls, the 69 Mpa and 300 TUs treatment appeared to outperform the other treatments.

Kokanee

Survival of controls (76.5%) exceeded that of other treatments by 14% or more. Only two treatments exceeded 50% eye up, including the 26°C: 20 MAF: 20 min duration (61.6%) and 28°C: 10 MAF: 10 min duration (52.2%) recipes (Table 9). For the three treatment temperatures (26, 27, & 28°C) that were conducted at 20 MAF and 20 min duration, survival decreased as temperature increased, with a sharp decline occurring at 28°C.

Induction rates approached or exceeded 90% for all but the two least intense treatments. For the three treatment temperatures (27, 28, & 29°C) that were conducted at 10 MAF and 10 min duration, induction rates increased as temperatures increased. The 27°C: 20 MAF: 20 min duration treatment produced the highest mean induction rate (98.3%). Because this treatment also resulted in a relatively high survival rate (48.7%), it appears to be the best treatment available at present.

Lake Trout

Survival of lake trout eggs and fry was still very low despite the use of treatments designed to be less intense than those used in 2001. Survival from egg to eyed egg was about 70% that of controls, and survival from egg to fry was about 16% of controls. This large drop in survival across stages was influenced by very high mortality during emergence from the eggshell. Although some slight improvements in survival occurred by reducing treatment intensity, the differences were small. For the 28°C treatments, mean survival from green to eyed

egg increased by 6% when MAF was reduced from 15 to 10 min. For the 29.4°C treatments, mean survival increased by 9% when the duration was decreased from 7 to 5 min. Survival within treatments for replicates one and two was nearly equal, but dropped on average about 25-30% for the third replicate, which was likely caused by poor egg quality. Although this drop in survival increased the associated error bound substantially, my ability to compare performance of the treatments was not influenced.

Due to low survival rates and a laboratory error, no induction rate information was available for the third replicate from all four treatments. Within treatments, induction rates for the second replicate were from 1.5 to 4 times higher than for the first replicate. The highest mean induction rate (62.5%) was provided by 29.4°C: 18 MAF: 7 min duration treatment.

DISCUSSION

Performance of Sterile Trout in High Mountain Lakes

Combined gill net and angling catch of diploid rainbow trout in 2002 from the pilot study lakes exceeded that of triploids by over four times. Combined with the catch statistics from 2001 (Figure 5; Kozfkay and Megargle 2001), the total catch of diploids was 2.5 times higher than for triploids over the two years of sampling. Even though these results are preliminary and were drawn from only three lakes within a small geographical area, the drastic performance difference is concerning, especially since hundreds of high mountain lakes have been stocked exclusively with triploid rainbow trout since 2001, using stocking densities that were based on past experience with diploid fish. Similarly poor performance was noted for coho salmon *O. kisutch* in Johnson Lake, Alaska (Rutz and Baer 1996). Relative catch frequencies for diploid and triploids were 56% and 44% in 1994 and 75% and 25% in 1995, respectively. In contrast, triploid brook trout outperformed diploid brook trout in an Adirondack lake due to reduced emigration of triploid females. A greater percentage of diploid brook trout (32%) emigrated from Mountain Pond, New York than triploids (17%; Warrillow et al. 1997). Warrillow et al. (1997) speculated that emigrants suffered higher mortality than lake residents, which reduced the availability of diploids to lake anglers.

The CPUE for gill net captures should have been an unbiased index of the relative abundance of the two test groups. During 2002, these data indicated that diploids (CPUE = 0.09) were three times more abundant than triploids (CPUE = 0.03). In contrast, comparison of the hook and line sampling data indicated that catch rates for diploids (CPUE = 0.35) exceeded those for triploids (CPUE = 0.03) by 12 times. Although this suggests that diploid rainbow trout were about four times more catchable than triploids, these observations are based on very limited sample sizes and should be considered preliminary. Data collected for the full-scale evaluation during 2004-2005 will allow a better comparison of the catchability of diploid and triploid trout in high mountain lakes.

Due to the small number of fish caught from each test group within lakes, length and weight measurements were highly variable and yielded wide confidence intervals, especially for triploids. The small sample sizes prevented any statistically valid comparisons regarding differences in length or weight between groups. However, mean length of diploids for all lakes ($n = 3$) was 7 mm longer than for triploids, similar to the results of 2001 (Kozfkay and Megargle 2001). The weight difference for 2002 approached 15 grams. This difference was slightly less than the 24 g difference observed in 2001. Apparently, triploids added more weight than diploids

from 2001 to 2002. Megargle and Teuscher (2000) saw similar trends in a comparison of diploid and triploid rainbow trout in a lowland reservoir. In Treasureton Reservoir, mean length of the two groups was equal at 24 months, but the diploid group was 100 g heavier. Eventually, this trend was reversed as diploid fish matured and put more energy into gonadal development and reproduction. I suggest the four pilot study lakes be monitored again in 2003 and perhaps 2004 to determine if weight gain eventually conforms to the results of Megargle and Teuscher (2000). The inclusion of 16 additional lakes in the full-scale study will enable a detailed evaluation of longevity and growth differences between triploid and diploid fish across a broader geographical area.

Grit dye was an efficient method for differentially batch marking diploid and triploid rainbow trout. Retention for both groups was near 90% for a period that exceeded one year. Other studies have had similar results. Retention of grit on juvenile steelhead was 100% for a 90-day period in circular tanks (Pauley and Trout 1988). Nielson (1990) reported that grit dye was retained in 90% of cutthroat trout recaptured from Bear Lake, ID-UT, and some fish retained marks for the entire study, up to 12 years. Only a slight retention difference, 5%, existed between the three colors of dye used. The disparity was likely caused by differences in size of the pigment granules (Phinney et al. 1967). Smaller granules do not penetrate the epidermis as deeply and are lost at higher rates than larger granules. Although marking and retention rates were sufficiently high to compare relative performance, I probably underestimated the true retention rate that would be experienced by fish in high mountain lakes. Nipping and erosion contributed to substantial loss of fins in the relatively crowded hatchery-rearing tanks. Since grit dye may be retained in fin integument, fin loss likely contributed to mark loss in some individuals.

Production of Sterile Trout

The use of pressure to create triploid hybrids at Henrys Lake provided higher induction (100%) and more consistent survival rates than thermal treatments tested previously. During 2000, induction rates for thermally-treated production lots averaged 65% (Megargle and Teuscher 2000). Experiments in 2001 produced individual replicates with 100% induction rates, but these always coincided with one replicate with much lower induction or unacceptable mortality rates (Kozfkay and Megargle 2001). If additional testing corroborates the results from 2002, a production-level pressure chamber would probably improve induction rates and, therefore, offer more genetic protection for Yellowstone cutthroat in Henrys Lake.

Although the number of eggs available at Henrys Lake is sufficient to meet stocking requests, the survival provided by this recipe still leaves considerable room for improvement. Survival to eye up in this recipe (42%) was 4% higher than for controls. The lower relative survival of controls was caused by either poor fertilization / early survival in controls or increased survival in treatments. It seems counterintuitive for an invasive treatment to provide higher survival than no treatment, but several researchers have also noted increased survival in triploid interspecific hybrids compared to diploid interspecific hybrids (Seeb et al. 1988; Scheerer and Thorgaard 1983; Scheerer et al. 1987). Therefore, it was possible that the treatment caused higher survival rates. Conversely, egg quality may have influenced this result. However, I used a large number of females ($n = 6$) and males ($n = 6$) to lower the probability of one or more individual(s) substantially lowering survival rates due to poor gamete quality. Additionally, I conducted experiments in the middle of the spawning season when egg quality is typically optimal, which also would have lowered this probability.

Although induction rates provided by pressure were higher than any past heat shock treatments, this experiment did not directly compare the performance of heat and pressure. Due to the availability of only one pressure chamber and one heat shock table for this experiment, I was unable to avoid pseudo-replication. Therefore, differences in survival and induction rates may have been caused by differing egg quality and not just treatment effects, despite our attempts to lower this probability by using a large number of adults and taking gametes during the middle of the spawning season. To ensure that improvements in survival and induction rates resulted from improved treatments and not variable gamete quality, additional equipment should be constructed or borrowed and a direct comparison of these treatments should be made in future years.

Over the range of temperatures examined for producing triploid kokanee, the best survival rates for eggs receiving heat shock treatments were generally 10-25% less than controls. For kokanee, survival was higher for 10 MAF and 10 min duration treatments than for 20 MAF and 20 min duration. Results from kokanee experiments indicated that at least three treatments provided similar induction (>95%) and survival rates (43-52%). The 27°C: 20 MAF: 20 min duration recipe provided slightly better performance than 28 or 29°C recipes, but slight modifications should be tested to determine if survival might be improved. Vander Haegen (1997) reported lower induction rates (87%) and very poor survival of kokanee (controls 50%; treatment 15%) treated with 26°C: 20 MAF: 20 min duration but speculated that water quality, not treatment effects, caused the high mortality.

Although refinement of the above-reported recipes may improve survival in the upcoming year, the positive results of 2002 (high induction rates & reasonable survival) suggest that field evaluation of sterile kokanee is now feasible. Rieman and Maiolie (1995) clearly demonstrated that kokanee vulnerability is strongly and positively related to total length and concluded that any method of producing larger fish should dramatically improve kokanee fisheries. Their model suggested that the vulnerability of 270 mm kokanee might be up to 20 times higher than for 230 mm fish. Because kokanee die at first spawning and sterilization may delay or eliminate spawning mortality, the possibility of producing larger, more vulnerable fish seems likely. I, therefore, recommend that IDFG undertake field experiments to evaluate longevity, growth, survival, and fisheries created by stocking paired groups of diploid and triploid kokanee in the field.

For brook trout, three treatments provided 100% induction rates with comparable survival to eye-up (50-62%). Among these treatments, the highest survival was provided with the 29.4°C, 18 MAF, and 7 min duration recipe (Tim Yesaki, British Columbia Ministry of the Environment, personal communication). The mean survival rate for this recipe was 12% higher than for the recipe most commonly cited in the literature, 28°C: 10 MAF: 10 min duration, for producing triploid brook trout (Sheerer and Thorgaard 1983; Warrillow et al. 1997). Since survival rates appeared to improve at shorter durations, additional testing should include even shorter durations, 4-5 min, to potentially reduce treatment induced mortality and approximate control levels (89%).

Despite two years of experiments and the testing of seven different thermal treatments that produced acceptable results with other species, no method for producing triploid lake trout with acceptable survival rates has been developed. During 2002, treatments were designed to be less intense to potentially reduce the high mortality rates experienced during 2001. Although some small survival improvements were noted, mortality was still excessive, especially as eggs approached and began to hatch (Dwight Aplanalp, IDFG, personal communication). Furthermore, reduced intensity in treatments lowered induction rates from that of 2001

experiments. So further reductions in temperature or duration would likely provide less than 95% induction rates.

Lake trout spawn at colder temperatures (MacLean et al. 1981) than all other species tested previously in Idaho. By using the same temperatures as used for other species, lake trout eggs may have received a relatively harsher shock. This caused immediate and delayed mortality. The only other reasonable alternative for producing large numbers of triploid lake trout is the use of hydrostatic pressure. Advantages of pressure treatment include the use of ambient water temperatures and that it has been shown to work in a closely-related species. For arctic char, 9,500 psi pressure treatments at 225 and 300°C MAF yielded 100% triploids with "excellent" survival (Keefe and Benfey 1995). Also, for arctic char, Gillet et al. (2001) produced 100% triploids with a 9,500 psi: 320°C MA: 5 minute duration treatment, and survival was approximately 90% that of controls. These recipes offer a good starting point for improving survival and induction rates for lake trout in future experiments.

RECOMMENDATIONS

1. Resample the pilot study lakes in 2003 to estimate potential longevity, relative survival, and growth differences between diploid and triploid rainbow trout.
2. Use thermal shock treatments of 29.4°C: 18 MAF: 7 min duration to produce triploid brook trout for Henrys Lake and design experiment to determine if survival may be improved with less intense treatments.
3. Directly compare heat and pressure treatment at Henrys Lake to induce triploidy in rainbow X Yellowstone cutthroat trout hybrids by using a minimal number of treatments and a higher number of replicates to ensure that the result of better performance in pressure shocked treatments was not influenced by varying egg quality.
4. Use thermal shock treatments of 27°C: 20 MAF: 20 min duration to produce triploid kokanee and design experiment to determine if survival may be improved with less intense treatments.
5. Evaluate longevity, growth, survival, and fisheries created by stocking paired groups of diploid and triploid kokanee in the field.
6. Design experiments to test the efficacy of pressure treatments to produce triploid lake trout.

ACKNOWLEDGMENTS

I would like to thank Dennis Lee and Steve Yundt for their field assistance during high mountain lake surveys. Paul Kline, Dan Baker, Brian Malaise, and Tom Kent provided rearing space, calculated rearing densities, and oversaw tank cleaning to allow assessment of grit retention in rainbow trout at Eagle Fish Hatchery. Damon Keane provided additional eggs for Henrys Lake hybrid and brook trout experiments. He also reared and enumerated the test groups. Rick Alsager, Dan Baker, Bob Turik, Travis Brown, Ken Felty, Shane Claborn, and Chuck Keister collected kokanee adults and spawned and shipped eggs for induction experiments. Phil Coonts, Mick Hoover, and Brian Grant developed rearing containers and fed and enumerated kokanee at Mackay Fish Hatchery. Dwight Aplanalp, Jeff Seggerman, Mark Olson, and Tom Kent picked lake trout eggs, installed rearing containers, and fed and enumerated test groups at Grace Fish Hatchery. Dan Schill provided direction and advice for these research projects and critically reviewed this manuscript.

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**ANNUAL PERFORMANCE REPORT
SUBPROJECT #3: PREDATOR TRAINING**

State of: Idaho

Grant No.: F-73-R-25, Fishery Research

Project No.: 4

Title: Hatchery Trout Evaluations

Subproject #3: Predator Training

Contract Period: July 1, 2002 to June 30, 2003

ABSTRACT

The ability of juvenile salmonids to learn to recognize predators and initiate avoidance behaviors in aquaria has been well established, but field evaluations are sparse. In this pilot study, I began research designed to test whether the survival and eventual return to creel rate of fingerling rainbow trout *Oncorhynchus mykiss* could be increased by exposing them to piscine predators prior to release. Adult rainbow trout were introduced into production raceways eight and nine at the Hagerman Fish Hatchery as predators. Control or untrained fingerlings were reared in two other production raceways. Five days after introduction of the predators, I examined the stomach contents of five predators from raceway eight. Two had empty stomachs. The stomachs from the other three predators contained six, eight, and nine fingerlings. The stomachs from 10 predators released in raceway nine were also examined. Three were empty. The stomach contents from the other seven predators contained from 7 to 20 fingerlings. Predators from both of the training raceways selected prey that was substantially shorter than the mean length of fingerlings found in the raceway. Equal numbers of trained and untrained fingerlings were stocked into Oakley, Magic, and CJ Strike reservoirs. With 124 hours of gill net effort in Oakley Reservoir, only one predator trained and one control fingerling were caught. Due to the poor catch rates and small size of fingerlings caught, no sampling was conducted at the other study sites. Due to an infectious hematopoietic necrosis outbreak during rearing of the test groups, the ability of this pilot study to compare relative survival was compromised. In conclusion, medium-sized rainbow trout were found to be an efficient predator for future studies, and they tended to consume only the smallest fish in the raceways.

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INTRODUCTION

A fish's ability to recognize predators is determined primarily by genetics and prior experience (Huntingford 1993). Prey species that evolved in predator-rich environments are able to recognize predators quickly and elicit predator avoidance strategies without prior exposure to predators (Johnson et al. 1993). Prey species that evolved in predator-poor environments seem to lack this innate ability but may learn to recognize predators after one or a series of attack(s) on conspecifics (Patten 1977). Learning is thought to occur through social communication, which is transferred by visual, olfactory, or other cues (Suboski et al. 1990).

By eliminating piscine and avian predation along with other causes of natural mortality, production fish hatcheries are able to supply large numbers of salmonids to habitats that would support few or no fisheries. However, by removing early life-history survival constraints, the behavior of stocked trout is altered from that of their wild counterparts (Berejikian et al. 1996; Dickson and MacCrimmon 1982). Hatchery trout are often more aggressive (Mesa 1991; Fenderson et al. 1968) and show less ability to recognize and react to predators (Berejikian 1995; Healey and Reinhardt 1995). These altered behavioral characteristics may explain, in part, why the survival rate of stocked trout is lower than that of wild trout or trout produced directly from wild parents (Fraser 1981; Miller 1951; Miller 1953).

Several researchers have trained naive prey to recognize predators and elicit avoidance behaviors. The survival rate of predator-conditioned coho salmon *Oncorhynchus kisutch* fry was 25% greater than the survival rate of naive fry when exposed to torrent sculpin *Cottus rhotheus* in artificial stream channels (Patten 1977). Similarly, juvenile coho salmon exposed to predation events from behind clear partitions were over twice as likely to avoid unrestrained lingcod *Ophiodon elongatus* than untrained salmon (Olla and Davis 1989). Thompson (1966) used an electrified fish model to train juvenile chinook salmon *O. tshawytscha*, and after stocking, found two and a half times more untrained fish than trained fish in the stomachs of piscine predators. Brown et al. (1997) demonstrated that naive fathead minnow *Pimephales promelas* learned to chemically recognize a predator, northern pike *Esox lucius*, in less than four days, but visual recognition did not occur until several days later. Rainbow trout do not possess the same alarm pheromones as cyprinids but appear able to recognize the scent of injured conspecifics and predators (Brown and Smith 1998).

Although the majority of the literature suggests a benefit to training naive prey, at least two researchers have concluded that predator training had no benefit. The use of an electrified loon *Gavia immer* model failed to increase the post release survival of brook trout *Salvelinus fontinalis* (Fraser 1974). He observed that conditioned fish moved 0.5 m laterally when the model approached and speculated that this behavior had no survival benefit. Berejikian et al. (1999) were able to train chinook and coho salmon to recognize potential predators in aquaria, but did not observe a post release survival improvement. They speculated that trained and untrained fish intermingled after stocking and that predator recognition and avoidance behaviors were passed from trained fish to untrained fish through social communication.

No studies designed to improve predator avoidance of rainbow trout on a production scale have been conducted but would be desirable, as the survival of fingerling rainbow trout in Idaho and elsewhere is often low. For instance, creel surveys conducted from November 1990 through 1992 indicated that less than 1% of fingerlings stocked in Cascade Reservoir returned to the creel (Dillon 1995). Similarly, the return to creel rate of fingerlings in Magic Reservoir is often very low. From 1992-1995, return to creel rates ranged from 0.1% to 5.8% (Teuscher

1998). Given such low return rates and the results of previous studies, increased post-release survival associated with predator training could dramatically increase the efficiency of the resident hatchery trout program in systems where predation limits survival.

RESEARCH GOAL

1. To increase the post-release survival and return to creel rate of rainbow trout stocked as fingerlings.

OBJECTIVES

1. To undertake a pilot study that within one year assesses survival advantages of predator training at the hatchery production scale.
2. In the next four years, evaluate whether post-stock survival of predator-trained fingerlings can exceed that of untrained fingerlings by 25% or more.

DESCRIPTION OF STUDY AREA

Study waters were selected based upon four criteria, including predator abundance, low probability of dewatering, recent stocking by Hagerman Fish Hatchery, and ability to recapture stocked fingerlings prior to reaching harvestable size. Oakley, Magic, and CJ Strike reservoirs were selected (Figure 7). Oakley Reservoir is an impoundment of Trapper and Goose Creeks, located about 26 miles south of Burley, Idaho. Oakley Reservoir supports an abundant population of walleye *Stizostedion vitreum*, yellow perch *Perca flavescens*, and rainbow trout. Magic Reservoir is an impoundment of the Big Wood River about 15 miles south of Bellevue, Idaho. Potential predators in Magic Reservoir include rainbow and brown trout *Salmo trutta* as well as yellow perch. CJ Strike Reservoir is a mainstem impoundment of the Snake River about 20 miles southwest of Mountain Home, Idaho. Potential predators include large- and smallmouth bass *Micropterus salmoides* and *M. dolomieu*, channel catfish *Ictalurus punctatus*, and rainbow trout.

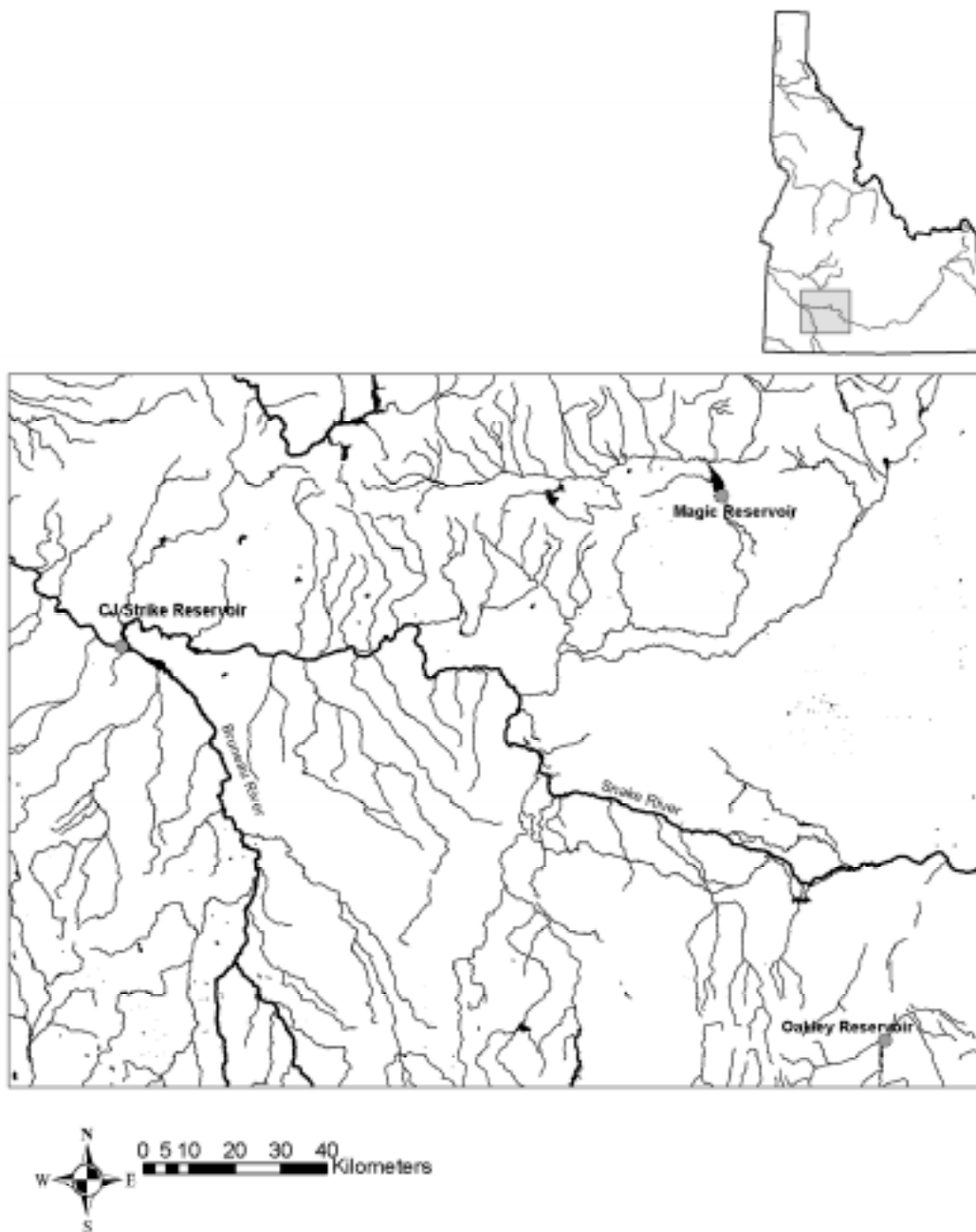


Figure 7. Location of the three reservoirs (Oakley, Magic, and CJ Strike) that were stocked with predator trained and untrained fingerlings during May 2002.

METHODS

Fish used for the predator training experiment were Hayspur and Kamloops strains of rainbow trout. Test fish were reared in four production raceways at Hagerman Fish Hatchery on Tucker Springs water. Raceways 4 and 7 were designated as controls and raceways 8 and 9 for predator training.

Fingerlings from each raceway were crowded and held for grit marking. On April 24 and 25, 2002, control fingerlings were marked with green grit dye and predator-trained fingerlings with red grit dye. Approximately 200 fingerlings were dipnetted at one time and placed in a wooden framed marking box with a wire mesh bottom. Grit dye was applied with a modified sandblasting gun using compressed air (460-490 kg/m²) as a propellant from a distance of about 30 cm. The process was repeated until the entire raceway was marked.

After the test groups recovered from marking stress (5-6 days), adult rainbow trout were transferred from the University of Idaho Experimental Station and placed into two experimental raceways. Each predator was measured to the nearest mm and weighed to the nearest gram. One predator was introduced for about every 5,000 fingerlings. On May 1, 2002, 10 predators were introduced into raceway 8 ($\bar{X} = 382.4 \pm 13.6$ mm, 853.4 ± 115.7 g), and 20 predators were introduced into raceway 9 ($\bar{X} = 390.5 \pm 9.8$ mm, 884.4 ± 84.7 g).

On May 6, I examined the stomach contents of five predators from raceway 8 and ten predators from raceway 9. Stomach contents were examined through forced regurgitation using a 30 cm catheter (1.5 cm diameter) and 300 cm³ syringe. The total number of ingested fingerlings was counted. Additionally, the length of whole fingerlings was measured to the nearest mm, and the length of partially digested fingerlings was approximated. No attempt was made to estimate the length of heavily digested prey. I tested the null hypothesis that predators selected fingerlings based on length in proportion to their availability. For each raceway separately, the null hypothesis was rejected if the 95% confidence intervals calculated for the mean difference in length between fingerlings found in the raceways and the stomachs of predators did not include zero (Zar 1996).

On May 13, 2002, a random sample of 100 fingerlings from each raceway was collected. Length was measured to the nearest millimeter and weight to the nearest gram. Grit marking rate was determined with a portable fluorescent lamp under a black plastic garbage bag. For each study water, approximately equal numbers of control and predator trained fingerlings were stocked. Fingerlings were loaded into one compartment of a tractor-trailer stocking unit and released through a discharge tube, except for Oakley Reservoir where fingerlings were stocked from a two-ton transport truck. On May 13 and 14, 2002, CJ Strike Reservoir was stocked with 100,000 fingerlings (i.e. 50,000 trained and 50,000 controls), Magic Reservoir with 120,000 fingerlings, and Oakley Reservoir with 40,000 fingerlings.

On September 10 and 11, 2002, I sampled Oakley reservoir with floating and sinking experimental gill nets. Floating and sinking gill nets measured 46 m long by 2 m deep and were comprised of 8 m panels of randomly placed 38, 51, 64, 76, 102, and 127 mm stretch mesh. Target species were measured to the nearest mm, weighed to the nearest gram, and examined for grit dye with a portable fluorescent lamp. Fifty individuals of each nontarget species were weighed and measured, and any additional individuals were counted. Data collected from nontarget species was provided to regional fisheries managers.

RESULTS

Of the five predators examined from raceway 8, two had empty stomachs. The stomachs from the other three predators contained six, eight, and nine fingerlings. Mean length of ingested fingerlings was 79.1 ± 7.6 mm. Of the 10 predators examined from raceway 9, three had empty stomachs. The other seven predators had from 7 to 20 fingerlings in their stomachs. The mean length of ingested fingerlings was 75.5 ± 2.9 mm. Predators from both of the training raceways selected fingerlings that were statistically shorter than the average found in that raceway. Mean length of available fingerlings in raceway 8 ($\bar{X} = 110.6 \pm 3.9$ mm) was 31.5 ± 8.3 mm longer than the mean length of fingerlings found in stomachs (Figure 8). Mean length of available fingerlings in raceway 9 ($\bar{X} = 111.9 \pm 4.0$ mm) was 36.3 ± 4.9 mm longer than the mean length of fingerlings found in stomachs (Figure 9).

Marking success on May 9, 2002, 14-15 days after impregnation of grit dye, equaled or exceeded 95% for all raceways. Mean length of fingerlings in the predator raceways (8 and 9; see previous paragraph) was equal. However, control fingerlings were shorter than the experimental fingerlings by about 5 mm in raceway 4 ($\bar{X} = 106.5 \pm 2.6$ mm) and 12 mm shorter in raceway 7 ($\bar{X} = 99.7 \pm 3.4$ mm).

In Oakley Reservoir, 118 rainbow trout were caught with 124 hours of gill net effort. Only one predator trained (242 mm; 150 g) and one control fingerling (244 mm; 145 g) were caught. Due to the poor catch rates for and small size of fingerlings in Oakley Reservoir, no sampling was conducted at the other two reservoirs.

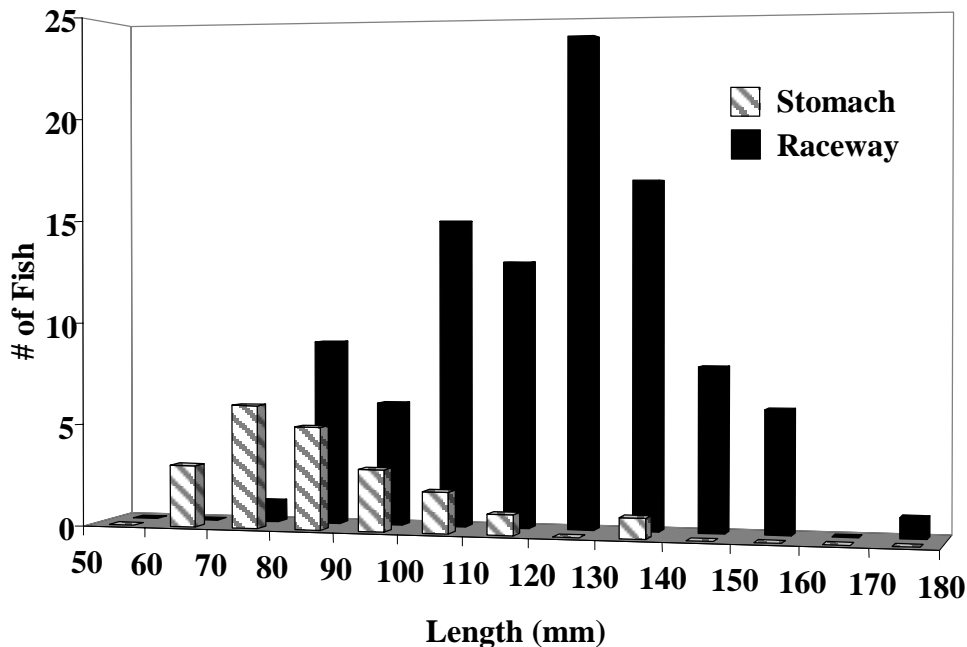


Figure 8. Length frequencies of fingerling rainbow trout available to predators ($n = 100$) in raceway 8 (black bars) and of those found in the stomachs of three predators ($n = 21$; white / gray bars).

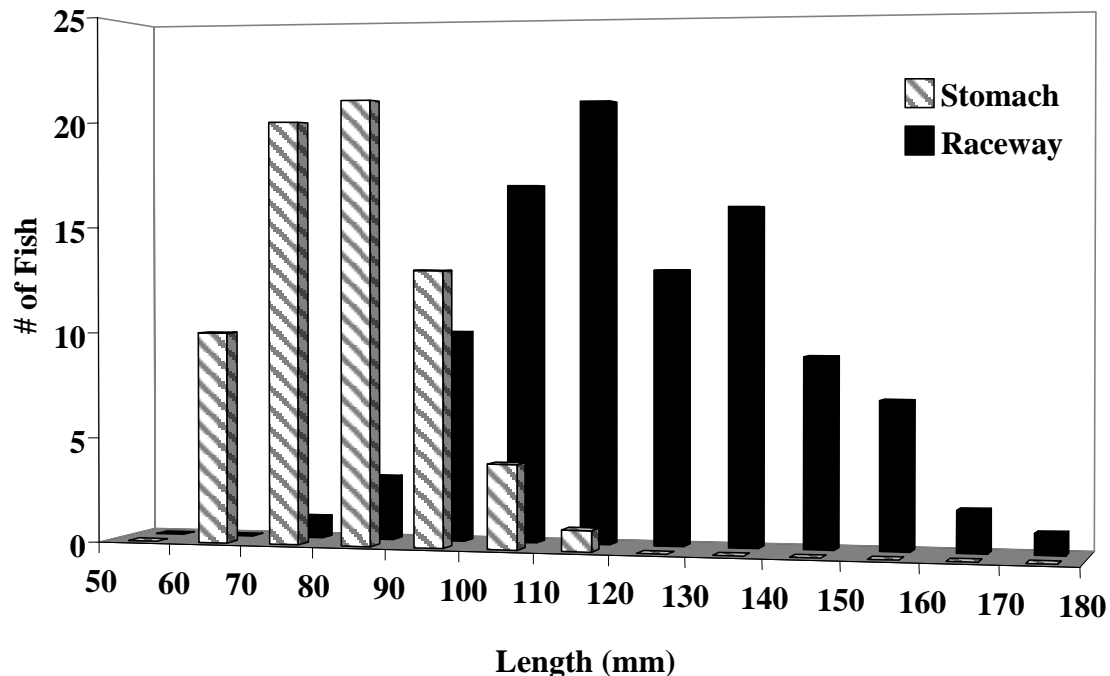


Figure 9. Length frequencies of fingerling rainbow trout available to predators (n = 100) in raceway 9 (black bars) and of those found in the stomachs of seven predators (n = 69; white / gray bars).

DISCUSSION

The ability of juvenile salmonids to learn to recognize predators and initiate avoidance behaviors in aquaria has been well established. Several different species of predators have been used. For the majority of these studies, predators were collected from the wild and transferred to laboratories. In the present study, I originally proposed to use several highly piscivorous species such as tiger musky *E. lucius* X *E. masquinongy* or bass *Micropterus* sp., but due to disease concerns rainbow trout from a certified disease-free source were used. There was some concern whether medium-sized rainbow trout would act as an efficient predator due to their previous diet (pellets), their relatively small size considering they had to ingest 75 mm and larger fingerlings, and anecdotal observations by hatchery personnel. According to these observations, when larger rainbow trout have accessed several hatcheries, they do not chase or consume fingerlings. This study contradicts these observations. Medium-sized adult rainbow trout were highly efficient at capturing fingerlings. In addition, they were observed to chase and strike at fingerlings on a regular basis.

To my knowledge, no attempts have been made to integrate experimental training methods into a production resident hatchery program. This may be due to the fact that introducing predators into raceways violates standard hatchery management principles; it creates a possibility for disease transfer and reduces total survival since some fingerlings are ingested. For this study, I sought to reduce the potential for disease transfer by using predators from a certified disease-free source. In future years, rearing predators at the station where training occurs may eliminate this concern. The ingestion of fingerlings during training reduced

the total number of fish available for stocking; however, the cost of training in terms of reducing the number of fish returned to the creel was likely minimal. Predators strongly selected the smaller individuals. If survival rates of fingerlings were directly related to size (Garvey et al. 1998; Miranda and Hubbard 1994; Santucci and Wahl 1993), the smaller fingerlings would have had poor post-release survival rates if they were stocked instead of being ingested. To estimate the cost of training in future years, I recommend that bioenergetics techniques be used to estimate the total number of fingerlings ingested during the training period.

Due to an infectious hematopoietic necrosis outbreak in two of the raceways during rearing of the test groups, the ability of this study to compare the relative survival of trained and untrained fingerling groups was compromised. The disease outbreak forced paired stockings of two strains of fish in each of the study waters (i.e., control Hayspur strain and predator trained Kamloops). Consequently, any differences in survival could not be solely attributed to treatment due to potential differences in strain performance (Megargle and Dillon 1994). Furthermore, trained fish were statistically larger than controls, which added another source of bias to comparisons of the test groups. Due to these potentially large sources of bias, I recommend that future plans for sampling the test waters be terminated as differential survival could not be attributed solely to whether a group was trained or not.

In conclusion, large rainbow trout were found to be an efficient predator for future studies; however, the current design prevented assessment of whether relative survival was improved through training. Despite this result, the above pilot study provided sufficient background and experience for me to recommend a larger scale predator training test using rainbow trout as predators during the upcoming field season.

RECOMMENDATIONS

1. Discontinue plans for further sampling of CJ Strike, Magic, and Oakley reservoirs.
2. Redesign study, and focus on stocking smaller reservoirs with equal length fingerlings of the same strain.

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